The role of starfish (*Asterias rubens L.*) predation in blue mussel (*Mytilus edulis* L.) seedbed stability

Antonio Agüera García

The role of starfish (*Asterias rubens* L.) predation in blue mussel (*Mytilus edulis*) seedbed stability

Antonio Agüera García

Thesis committee

Promotor

Prof. Dr A.C. Smaal
Professor of Sustainable Shellfish Culture
Wageningen University

Co-promotors

Dr J.M. Jansen
Advisor, Nederlandse Aardolie Maatschappij B.V., Assen

Dr T. Schellekens
Quantitative Ecologist, Wageningen IMARES, Yerseke

This research was conducted under the auspices of the Graduate School of Wageningen Institute of Animal Sciences (WIAS).

The role of starfish (*Asterias rubens* L.) predation in blue mussel (*Mytilus edulis*) seedbed stability

Antonio Agüera García

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 13 February 2015
at 11 a.m. in the Aula.

Antonio Agüera García
The role of the starfish (*Asterias rubens* L.) predation in blue mussel (*Mytilus edulis* L.) seedbed stability.
170 pages.

PhD thesis, Wageningen University, Wageningen, NL (2015)
With references, with summaries in Dutch, English and Spanish

ISBN 978-94-6257-216-4

A mis padres

y hermanos

Contents

Chapter 1

General Introduction

In the Wadden Sea subtidal mussel beds are an important ecological component. Mussels are dominant habitat forming organisms and their offspring settle anywhere they find a suitable substrate, forming dense aggregations (seedbeds). Massive recruitment events result in formation of seedbeds composed exclusively by young mussels. Mussel beds provide substrate, shelter and are a food source for several organisms (Thiel and Ullrich 2002, Silliman et al. 2011). As such, mussels support a community of organisms that profit from them as habitat and food (Thiel and Ullrich 2002). Mussel beds have the capacity to enhance biodiversity, as it happens when they occur on soft substrates (Norling and Kautsky 2008, Buschbaum et al. 2008) such as the Wadden Sea. Subtidal mussel beds in the European Wadden Sea support a richer community than the alternative sediment bottoms (Buschbaum et al. 2008, Drent and Dekker 2014). Subtidal mussel beds are also a reliable food source for several organisms including birds, such as Eider ducks (Kats 2007). Besides the ecological importance as biodiversity enhancers and food source, mussel seedbeds in the Wadden Sea are an important economic resource for the Dutch aquaculture industry. Seedbeds are harvested in autumn and in spring and young mussels are transported to culture lots where the mussels grow until they reach market size (Smaal 2002). However subtidal mussel seedbeds may disappear within months after formation, even before they can be located or harvested.

Juvenile stages are the most vulnerable phase of bivalves benthic life (Barbeau et al. 1996). In sedimentary systems, mussel seedbeds are sensitive to perturbations caused by changes in environmental conditions: erosion and sedimentation during storms (Caceres-Martinez et al. 1994), temperature (Caceres-Martinez et al. 1994, Freitas et al. 2007, Jansen et al. 2009), salinity changes (Westerbom et al. 2008) and intraspecific competition (O'Neill et al. 1983, Petraitis 1995). Seedbeds are also subject to predation (Kamermans et al. 2009).

The problem of predation is not new, fisheries management, aquaculture and conservation programs focus on predation as a factor controlling bivalve juvenile survival (Barbeau et al. 1996, Gallagher et al. 2008, Kamermans et al. 2009). In the Wadden Sea predation by benthic fauna and diving ducks are thought to influence survival of subtidal mussel seedbeds (Smaal et al. 2014). Predation on seedbeds is highest in the first year after settlement (Navarrete and Menge 1996) and has an important role in the survival of seedbeds (Smaal et al. 2014). Predation impact on seedbeds is determined by the mussel mortality they cause, which in turn depends on the abundance of predators and their individual capacity to remove prey. In mussel beds, it is thought that survival and evolution of the bed benefit from low predation stress, as predators will remove weak individuals and ease intraspecific competition (Petraitis 1995, Gascoigne et al. 2005). In contrast, when predation pressure is higher, it causes damage to the mussel bed and jeopardize the stability of the bed by reducing individual density significantly and affecting the structure (Robles et al. 2009).

Figure 1.1. Several starfish feeding on mussels at a seedbed the Wadden Sea (photo by Michel Trommelen, 2009).

The common starfish (*Asterias rubens*) is known to consume substantial amounts of mussel seed (Kamermans et al. 2009) (Figure 1.1). *A. rubens* plays a key role in structuring many marine ecosystems (Menge 1982, Uthicke et al. 2009), being capable of limiting the spatial distribution of many of their prey species (Sloan and

Aldridge 1981, Dare 1982) including mussels in the Wadden Sea (Saier 2001) where high densities of starfish are commonly recorded (van Stralen et al. 2005). Among other characteristics, starfish populations undergo rapid changes in density, occasionally forming very dense aggregations that sometimes result in formation of swarming events and feeding fronts (Brun 1968, Sloan and Aldridge 1981, Dare 1982, Saier 2001). These swarms take place in the subtidal (Anger et al. 1977, Dare 1982, Allen 1983, Guillou 1996) and have disastrous consequences for natural mussel beds (Dare 1982) and bottom culture plots (Gallagher et al. 2008). Starfish swarms typically wipe out all available resources to the point of jeopardizing the sustainability of their own population (Guillou 1996).

Due to their dual value, ecological and economic, subtidal mussel seedbeds are recorded every year and classified within a stability range. The stability of subtidal mussel beds in the Wadden Sea is defined as the probability of the bed to survive its first winter, given average environmental conditions (Brinkman et al. 2003). The determination of the stability of a young mussel bed is the basis of management of mussel seed fishery and mussel bed restoration. The stability of a mussel bed is classified on a scale from one to five, with one being the most unstable (it will very likely disappear during winter) and five the most stable (will likely survive the winter). Unstable mussel beds are fished before winter and stable beds are left to mature prior to spring fishery; since 2009 some areas are closed to fishery with the intention to restore the area covered by mussel beds in the Wadden Sea and its associated biodiversity.

Stability classification is based on expert judgment by fishermen and scientists. Expert judgment is linked with the results from calculations of a habitat suitability map for mussel beds, which is based on mussel bed specific data, and measurements of abiotic and biotic parameters. The habitat suitability map indicates the probability of occurring mussel beds to survive the winter (Brinkman et al. 2003, Smaal et al. 2014, Figure 1.2).

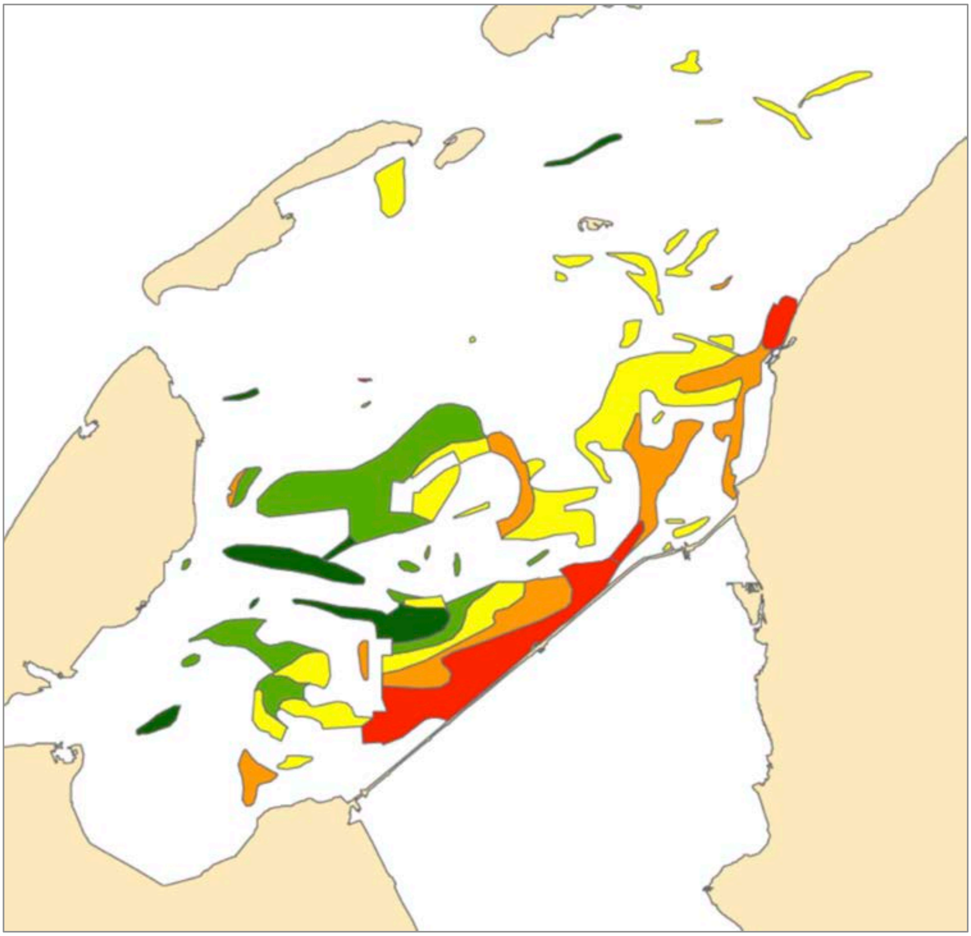


Figure 1.2. Stability map based on previous experience and expert judgement. Green – category 1, relatively unstable; light green – category 2; yellow – category 3; orange – category 4, red – category 5, relatively stable.

1.1 Ecophysiology of the common starfish, *Asterias rubens* L.

The Wadden Sea is a challenging environment for the organisms inhabiting it. The Wadden Sea has estuarine characteristics where temperature exhibits a large seasonal variability and is close to zero during winter, ice formation is also a common phenomenon in the Wadden Sea. In the Wadden Sea salinity has a marked spatial gradient, which also links to large tidal fluctuations and even larger seasonal changes (Zimmerman 1976, van Aken 2008). Salinity is mainly regulated by freshwater input from the IJsselmeer, that is controlled by sluices (Zimmerman 1976). Moreover the

Wadden Sea is a tidal embayment and tidal movements result in strong tidal flows. Flow velocities over 50 cm s^{-1} are common for almost any location within the Wadden Sea (Zimmerman 1976).

Environmental conditions such as temperature, salinity and hydrodynamic regime are known to affect distribution, abundance and performance of *A. rubens.* These environmental variables affect the impact of *A. rubens,* determining its predation capacity and affecting or controlling several steps during their life cycle. Thus environmental factors can limit keystone predation (Menge et al. 1994).

To fully understand and predict the role of *A. rubens* in the stability of mussel seedbeds it is mandatory to understand the role of environmental conditions in the performance of *A. rubens* as a mussel predator.

1.1.1 The effect of temperature on reproduction, growth and predation

Environmental temperature is especially important in ectotherms as they cannot regulate their own body temperature. In the case of *A. rubens*, as it happens with other starfish species, environmental temperature control breeding timing, larval development and seasonal activity levels; affecting *A. rubens* performance, limiting feeding and abundance.

Reproduction of *A. rubens* is limited to one annual breeding season. Spawning is controlled by the rise of temperature during spring (Briggs 1983, Joly-Turquin et al. 2013). In the Wadden Sea, reproduction takes places around April-May (Oudejans et al. 1979). Temperature also affects larval survival with larvae suffering an abnormal development at low temperatures (below 2ºC) (Benitez Villalobos et al. 2006).

In starfish, activity is limited by environmental temperature (MacKenzie 1969, Barbeau and Scheibling 1994). During winter *A. rubens* activity decreases and predation rate is lower and in some cases of little importance during that season (Hancock 1955). Feeding rate reflects directly on starfish individual growth and has a further effect on the population biomass (Witman et al. 2003). Low winter activity could be influenced by the photoperiod reduction during winter, as starfish have been suggested to exhibit enhanced diurnal feeding (Anger et al. 1977) and clear reactions to light (Castilla and Crisp 1970). However, *A. rubens* feeding rate on mussel seed could be significantly higher at the end of summer and autumn, in an attempt to increase reserves before temperatures become too low (Jangoux and Impe 1977, Gaymer et al. 2001). However the decrease of predation on mussels with seasonal temperature and photoperiod has never been quantified. High temperatures (over 20ºC) can cause mortality of adult individuals (Smith 1940).

1.1.2 Performance of *Asterias rubens* under osmotic stress

Starfish are osmoconformers and changes of environmental ionic concentration affect their performance, distribution and abundance. *A. rubens* tolerance towards environmental salinity changes is limited by their acclimation capacities (Saranchova 2001). Local acclimation and poor capacity to tolerate salinity changes are especially important when the environment is as variable as the Wadden Sea.

Sudden or gradual changes in salinity affect spawning events of *A. rubens* (Nauen 1978), yet reproducing populations exists down to salinities of 8 PSU (Kowalski 1955). Osmotic stress affects egg and larval development (Saranchova and Flyachinskaya 2001) reducing viability and causing important mortality at those stages. Osmotic stress causes important mortality events of adult individuals (Binyon 1961, Shumway 1977, Berger and Naumov 1996). Besides mortality, osmotic stress also has an impact on mobility (Castilla and Crisp 1973, Barker and Russell 2008), affecting individuals capacity to attach to the substrate (Berger and Naumov 1996).

By inducing mortality, salinity changes could determine *A. rubens* spatial and temporal distribution. By affecting capacity to move and attachment, salinity may limit predation impact of the present population. In the Wadden Sea freshwater discharge from the IJsselmeer, anthropologically controlled, is the main factor controlling the spatial variation in salinity, that may have a profound effect in the distribution, abundance and predation performance of *A. rubens*, especially during winter rains or spring snow melts.

1.1.3 Physical limitations imposed by high velocity flows

The impact of hydrodynamics in distribution of organisms is well documented. Constant exposure to a high hydrodynamic regime caused by swell, tidal currents or frequent storms has a direct effect in structuring communities (Gagnon et al. 2003). High swell and high flows force those organisms to take refuge and reduce the time spent searching and handling prey. Whereas starfish can move safely on hard substrates, even when they are exposed to strong hydrodynamics (Hennebert et al. 2010), their locomotion and attachment system is ineffective on non-cohesive soft substrates (Anger et al. 1977). Thus, their only mechanism to avoid dislodgement from soft sediments by currents or waves seems to be by seeking sheltering. Storms and tidal currents can dislodge starfish individuals, with great impact on densities (Anger et al. 1977), or prevent the establishment of new recruits. Storms could also have an effect on disaggregating feeding fronts (Dare 1982). As mobile animals, starfish are capable of adjusting their behaviour to minimize the impact of high-energy environments. Under high stress conditions, starfish mobility is reduced. This has a direct effect on its capacities as a predator as the time spent searching for and handling food is reduced (Rochette et al. 1994, Gagnon et al. 2003). It remains unknown how *Asterias spp.* endure a highly dynamic environment while inhabiting mussel beds on soft bottoms and to what extent hydrodynamics will limit their activity levels and predation performance.

1.2 *Asterias rubens* and *Mytilus edulis* predator-prey interaction.

Understanding the interaction of predators with their prey and with other predators, conspecifics or not, is essential to assess and predict the effects that particular predator may have on the ecosystem. Starfish are selective foragers showing a marked change in the use of prey resources with increasing size (ontogenetic niche shift) (Himmelman et al. 2005). Starfish predation on mussels changes in two ways: size selection, accommodated mainly by the access to more profitable food resources when they get bigger; and feeding rates, as starfish are known to exhibit a reduction of feeding rate per animal wet weight when they grow (Anger et al. 1977, O'Neill et al. 1983). Starfish prey size is limited by its own size, and a correlation between prey size selection and starfish arm length has been observed (Anger et al. 1977, Dolmer 1998). Therefore as a starfish grows it will be able to feed on a wider mussel size spectrum, having access to bigger specimens. In some cases mussels may overgrow a starfish’s maximum prey size and attain size refuge (Dolmer 1998).

Prey selection is not only determined by prey energetic value. Prey dynamics have an effect on prey availability and will result in a trade-off between costs of searching for most valuable prey and prey value (Wong et al. 2006) and as such prey dynamics will affect prey selection and predation rates. Mussels associate with other mussels, forming dense matrices (Buschbaum et al. 2008), this association is thought to serve as a defence mechanism making predation more difficult, and affecting the size of prey selected.

1.3 Aim and research questions

This thesis aimed at **determining the role of *A. rubens* in development and survival of mussel seedbeds.** There exists a vast literature on *A. rubens* biology and physiology. However most available research is either focused on *A. rubens* population dynamics without consideration of environmental conditions or on the physiological response of *A. rubens* to temperature or salinity changes without considering the interaction with its preys. There is no literature quantifying the effects of seasonal temperature changes or salinity variations on starfish predation rate on mussels. The same is true for the effect of hydrodynamics. Moreover, although *A. rubens* is a well known mussel predator, the effect of mussel aggregation on feeding rates and predator behaviour have never been researched. The research presented here focuses on the effects of environmental conditions in the interaction of *A. rubens* with the mussel, quantifying predation rates and performance of *A. rubens* when inhabiting and feeding on mussel seedbeds under challenging environmental conditions.

In line with the aim of this thesis, the research work sought answers to the following four research questions:

1. How important is *A. rubens* predation during winter? What is the role of temperature?
2. How is osmotic stress affecting *A. rubens* feeding rate?
3. How does *A. rubens* perform under high velocity flows? Does it affect its feeding rate?
4. How does mussel association with conspecifics affect *A. rubens* feeding rate?

To fulfil the objective of this thesis, answers to these questions were combined with previous literature knowledge and used in the formulation of a simulation model able to offer an overview of the potential impact of *A. rubens* on mussel seedbeds in the

Wadden Sea. This model could be used by the managing authorities to assess the impact of *A. rubens* in a particular mussel bed, whether it is natural, culture or a seed collector.

1.4 Thesis outline

The introduction, Chapter 1, stated the applied research question alongside with previous knowledge on the topic and background information. The aim is defined and the outline of the thesis presented.

Asterias rubens feeding rate from autumn until spring was quantified in Chapter 2. By means of a mesocosm experiment, *A. rubens* was exposed to the natural temperature change during seasons and its predation rate on mussels quantified, allowing us to gave an answer to question 1. Temperature and shading (light intensity) effect on observed feeding rates were assessed.

Chapter 3 sought the answer to question 2. In this chapter the effect of osmotic stress caused by salinity decreases of different intensity on *A. rubens* mortality and predation performance were studied. Osmotic stress affects activity, predation and survival, however when salinity change is not lethal, slow acclimation to the new situation takes place.

The effect of flow on *A. rubens* performance is studied in Chapter 4, providing an answer to question 3. In this chapter the capacity of *A. rubens* to endure high velocity flows and the effect on predation were assessed. Moreover starfish dependence on the capacity of mussel to ameliorate flow was studied. *A. rubens* is affected by flow velocity, reducing its activity at high flows, however flow amelioration by mussel clumps reduces the stress.

Chapter 5 continues on how mussel association with conspecifics reflects on starfish performance and behaviour giving necessary insight to answer question 4. In this chapter, the effect of clumping on how *A. rubens* approach mussel prey was studied. As mussel density increases, so does the complexity of its association with other mussels (larger and tighter clumps), this mussel defensive behaviour produces changes in prey selection by *A. rubens*.

Chapter 6 describes a simulation model integrating previous chapter results and available literature. This simulation model aims to assess the impact of a given scenario (mussel and starfish densities) under determined environmental conditions.

The final chapter, Chapter 7, contains the general discussion of the presented research. It discusses the overall results and focuses on the role of environmental conditions on the distribution and performance of *A. rubens* in the Wadden Sea. It finally addresses the application of the research question with an overview of the impact of *A. rubens* on mussel seedbed stability, the role of environmental conditions and predator and prey population structure.

References

Allen, P. L. 1983. Feeding Behaviour of *Asterias rubens* (L.) on soft bottom bivalves: a study in selective predation. The Journal of Experimental Marine Biology and Ecology 70:79–90.

Anger, K., U. Rogal, G. Schriever, and C. Valentin. 1977. In-situ investigations on the echinoderm *Asterias rubens* as a predator of soft-bottom communities in the western Baltic Sea. Helgoländer Wissenschaftliche Meeresuntersuchungen 29:439–459.

Barbeau, M. A., and R. E. Scheibling. 1994. Temperature effects on predation of juvenile sea scallops [*Placopecten magellanicus* (Gmelin)] by sea stars (*Asterias vulgaris* Verrill) and crabs (*Cancer irroratus* Say). The Journal of Experimental Marine Biology and Ecology 182:27–47.

Barbeau, M. A., B. G. Hatcher, R. E. Scheibling, A. W. Hennigar, L. H. Taylor, and A. C. Risk. 1996. Dynamics of juvenile sea scallop (Placopecten magellanicus) and their predators in bottom seeding trials in Lunenburg Bay, Nova Scotia. Canadian Journal of Fisheries and Aquatic Sciences 53:2494–2512.

Barker, M. F., and M. Russell. 2008. The distribution and behaviour of Patiriella mortenseni and P. regularis in the extreme hyposaline conditions of the Southern New Zealand Fiords. The Journal of Experimental Marine Biology and Ecology 355:76–84.

Benitez Villalobos, F., P. A. Tyler, and C. M. Young. 2006. Temperature and pressure tolerance of embryos and larvae of the Atlantic seastars *Asterias rubens* and *Marthasterias glacialis* (Echinodermata: Asteroidea): potential for deep-sea invasion. Marine Ecology Progress Series 314:109–117.

Berger, V. J., and A. D. Naumov. 1996. Influencia of salinity on the ability of starfishes *Asterias rubens* L. to attach to substrate. Biologiya Morya-Marine Biology 22:99–101.

Binyon, J. 1961. Salinity Tolerance and Permeability to Water of the Starfish *Asterias rubens* L. Journal Of The Marine Biological Association Of The United Kingdom 41:161–174.

Briggs, C. F. 1983, February. A study of some sublittoral populations of Asterias rubens (L.) and their prey. Port Erin. Ph.D Thesis. Liverpool University

Brinkman, A. G., T. Bult, N. Dankers, A. Meijboom, M. van Stralen, and J. de Vlas. 2003. Mosselbanken : Kenmerken , Oppervlaktebepaling en Beoordeling van Stabiliteit. Wageningen Alterra. Report 707. Pages 70.

Brun, E. 1968. Extreme population density of the starfish *Asterias rubens* on a bed of Iceland scallops. Astarte 32:1–3.

Buschbaum, C., S. Dittmann, J.-S. Hong, I.-S. Hwang, M. Strasser, M. Thiel, N. Valdivia, S.-P. Yoon, and K. Reise. 2008. Mytilid mussels: global habitat engineers in coastal sediments. Helgoland Marine Research 63:47–58.

Caceres-Martinez, J., J. Robledo, and A. Figueras. 1994. Settlement and post-larvae behaviour of *Mytitus galloprovincialis*: field and laboratory experiments. Marine Ecology Progress Series 112:107–117.

Castilla, J. C., and D. J. Crisp. 1970. Responses of *Asterias rubens* to olfactory stimuli. Journal Of The Marine Biological Association Of The United Kingdom 50:829–847.

Castilla, J. C., and D. J. Crisp. 1973. Responses of *Asterias rubens* to water currents and their modification by certain environmental factors. Netherlands Journal of Sea Research 7:171–190.

Dare, P. J. 1982. Notes on the swarming behaviour and population density of *Asterias rubens* L. (Echinodermata: Asteroidea) feeding on the mussel, *Mytilus edulis* L. Journal du Conseil 40:112–118.

Dolmer, P. 1998. The interactions between bed structure of *Mytilus edulis* L. and the predator *Asterias rubens* L. The Journal of Experimental Marine Biology and Ecology 228:137–150.

Drent, J., and R. Dekker. 2014. Macrofauna associated with mussels, *Mytilus edulis* L., in the subtidal of the western Dutch Wadden Sea. NIOZ Royal Netherlands Institute for Sea Research, Texel. Report 2013-7. Pages 77.

Freitas, V., J. Campos, M. Fonds, and H. W. Van der Veer. 2007. Potential impact of temperature change on epibenthic predator–bivalve prey interactions in temperate estuaries.

Gagnon, P., G. Wagner, and J. H. Himmelman. 2003. Use of a wave tank to study the effects of water motion and algal movement on the displacement of the sea star *Asterias vulgaris* towards its prey. Marine Ecology Progress Series 258:125–132.

Gallagher, T., C. A. Richardson, R. Seed, and T. Jones. 2008. The Seasonal movement and abundance of the starfish *Asterias rubens* in relation to mussel farming practive: a case study from the Menai Strait, UK. Journal of Shellfish Research 27:1209–1215.

Gascoigne, J. C., H. A. Beadman, C. Saurel, and M. J. Kaiser. 2005. Density dependence, spatial scale and patterning in sessile biota. Oecologia 145:371–381.

Gaymer, C. F., J. H. Himmelman, and L. E. Johnson. 2001. Distribution and feeding ecology of the seastars Leptasterias polaris and Asterias vulgaris in the northern Gulf of St Lawrence, Canada. Journal Of The Marine Biological Association Of The United Kingdom 81:827–843.

Guillou, M. 1996. Biotic and abiotic interactions controlling starfish outbreaks in the Bay of Douarnenez, Brittany, France. Oceanologica Acta 19:415–420.

Hancock, D. A. 1955. The Feeding Behaviour of Starfish on Essex Oyster beds. Journal Of The Marine Biological Association Of The United Kingdom 34:313–331.

Hennebert, E., D. Haesaerts, P. Dubois, and P. Flammang. 2010. Evaluation of the different forces brought into play during tube foot activities in sea stars. The Journal of Experimental Biology 213:1162–1174.

Himmelman, J. H., C. Dutil, and C. F. Gaymer. 2005. Foraging behavior and activity budgets of sea stars on a subtidal sediment bottom community. The Journal of Experimental Marine Biology and Ecology 322:153–165.

Jangoux, M., and E. V. Impe. 1977. The annual pyloric cycle of *Asterias rubens* L. (ECHINODERMATA: ASTEROIDEA). The Journal of Experimental Marine Biology and Ecology 30:165–184.

Jansen, J. M., H. Hummel, and S. W. Bonga. 2009. The respiratory capacity of marine mussels (Mytilus galloprovincialis) in relation to the high temperature threshold. Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 153:399–402.

Joly-Turquin, G., P. Dubois, S. Leyzour, P. Pernet, F. de Ridder, R. Pintelon, and M. Guillou. 2013. Contrasting relationships between pyloric caecum and gonad growth in the starfish Asterias rubens: combined field and experimental approaches. Journal of the ….

Kamermans, P., M. Blankendaal, and J. Perdon. 2009. Predation of shore crabs (*Carcinus maenas* (L.)) and starfish (*Asterias rubens* L.) on blue mussel (*Mytilus edulis* L.) seed from wild sources and spat collectors. Aquaculture 290:256–262.

Kats, R. K. H. 2007. Common Eiders Somateria mollissima in the Netherlands: the rise and fall of breeding and wintering populations in relation to stocks of shellfish.:336–336.

Kowalski, R. 1955. Untersuchungen zur Biologie des Seesternes Asterias rubens L. in Brackwasser. Kieler Meeresforschungen XI.

MacKenzie, C. L. 1969. Feeding Rates of Starfish, *Asterias forbesi* (Desor), at controlled water temperatures and during different seasons of the year. Fishery Bulletin 65:67–72.

Menge, B. A. 1982. Effects of feeding on the environment: Asteroidea. Pages 521–551 *in* M. Jangoux, J. M. Lawrence, and J. M. Lawrence, editors. Echinoderm Nutrition. A.A. Balkema, Rotterdam.

Menge, B. A., and J. P. Sutherland. 1976. Species diversity gradients: Synthesis of the roles of predation, competition and temporal heterogeneity. The American Naturalist 110:351–369.

Menge, B. A., E. L. Berlow, C. A. Blanchette, S. A. Navarrete, and S. B. Yamada. 1994. The Keystone Species Concept : Variation in Interaction Strength in a Rocky Intertidal Habitat. Ecological Monographs 64:250–286.

Nauen, C. E. 1978. The growth of the sea star, *Asterias rubens*, and its role as benthic predator in Kiel Bay. Kieler Meeresforschungen 4:68–81.

Navarrete, S., and B. A. Menge. 1996. Keystone Predation and Interaction Strength: Interactive Effects of Predators on Their Main Prey. Ecological Monographs 66:409–429.

Norling, P., and N. Kautsky. 2008. Patchy mussel (Mytilus edulis) communities as islands of high biodiversity in subtidal sediment habitats in the Baltic Sea. Aquatic Biology 4:75–87.

O'Neill, S., A. Sutterlin, and D. Aggett. 1983. The effects of size-selective feeding by starfish (*Asterias vulgaris*) on the production of mussels (*Mytilus edulis*) cultured on nets. Aquaculture 35:211–220.

Oudejans, R. C. H. M., I. V. D. Sluis, and A. J. V. Plas. 1979. Changes in the Biochemical Composition of the Pyloric Caeca of Female Seastars , *Asterias rubens* , During Their Annual Reproductive Cycle. Marine Biology 53:231–238.

Petraitis, P. S. 1995. The Role of Growth in Maintaining Spatial Dominance by Mussels (*Mytilus edulis*). Ecology 76:1337–1346.

Robles, C. D., R. A. Desharnais, C. Garza, M. J. Donahue, and C. A. Martinez. 2009. Complex equilibria in the maintenance of boundaries: experiments with mussel beds. Ecology 90:985–995.

Rochette, R., J.-F. Hamel, and J. H. Himmelman. 1994. Foraging strategy of the asteroid *Leptasterias polaris*: role of prey odors, current and feeding status. Marine Ecology Progress Series 106:93–100.

Saier, B. 2001. Direct and indirect effects of seastars *Asterias rubens* on mussel beds (*Mytilus edulis*) in the Wadden Sea. Journal of Sea Research 46:29–42.

Saranchova, O. L. 2001. Research into tolerance for the environment salinity in sea starfish *Asterias rubens* L. from populations of the White Sea and Barentz Sea. The Journal of Experimental Marine Biology and Ecology 264:15–28.

Saranchova, O. L., and L. P. Flyachinskaya. 2001. The Influence of Salinity on Early Ontogeny of the Mussel *Mytilus edulis* and the Starfish *Asterias rubens* from the White Sea. Russian Journal of Marine Biology 27:87–93.

Shumway, S. E. 1977. The effects of fluctuating salinities on four species of asteroid echinoderms. Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 58A:177–179.

Silliman, B. R., Silliman, B. R., M. D. Bertness, A. H. Altieri, J. N. Griffin, M. C. Bazterrica, F. J. Hidalgo, C. M. Crain, and M. V. Reyna. 2011. Whole-Community Facilitation Regulates Biodiversity on Patagonian Rocky Shores. PLoS ONE 6:e24502.

Sloan, N. A., and T. H. Aldridge. 1981. Observations on an aggregation of the starfish *Asterias rubens* L. in Morecambe Bay, Lancashire, England. Journal of Natural History 15:407–418.

Smaal, A. C. 2002. European mussel cultivation along the Atlantic coast : production status , problems and perspectives. Hydrobiologia 484:89–98.

Smaal, A. C., A. G. Brinkman, T. Schellekens, J. M. Jansen, A. Agüera, and M. R. van Stralen. 2014. Ontwikkeling en stabiliteit van sublitorale mosselbanken. IMARES Wageningen UR, Yerseke. Report C066.14. Pages 32

Smith, G. F. M. 1940. Factors limiting distribution and size in the starfish. Journal of the Fisheries Research Board of Canada 5:84–103.

Thiel, M., and N. Ullrich. 2002. Hard rock versus soft bottom: the fauna associated with intertidal mussel beds on hard bottoms along the coast of Chile, and considerations on the functional role of mussel beds. Helgoland Marine Research 56:21–30.

Uthicke, S., B. Schaffelke, and M. Byrne. 2009. A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. Ecological Monographs 79:3–24.

van Aken, H. M. 2008. Variability of the salinity in the western Wadden Sea on tidal to centennial time scales. Journal of Sea Research 59:121–132.

van Stralen, M., J. Craeymeersch, and J. Jol. 2005. Inventarisatie van het wilde mosselbestand in de Waddenzee in het najaar van 2005. Report 2005.48.2. Pages 19.

Westerbom, M., O. Mustonen, and M. Kilpi. 2008. Distribution of a marginal population of Mytilus edulis: responses to biotic and abiotic processes at different spatial scales. Marine Biology 153:1153–1164.

Witman, J. D., S. J. Genovese, J. F. Bruno, J. W. McLaughlin, and B. I. Pavlin. 2003. Massive Prey Recruitment and the Control of Rocky Subtidal Communities on Large Spatial Scales. Ecological Monographs 73:441–462.

Wong, M. C., M. A. Barbeau, M. Dowd, and K. R. Richard. 2006. Behavioural mechanisms underlying functional response of sea stars *Asterias vulgaris* preying on juvenile sea scallops *Placopecten magellanicus*. Marine Ecology Progress Series 317:75–86.

Zimmerman, J. T. F. 1976. Mixing and flushing of tidal embayments in the western Dutch Wadden Sea. Part I: Distribution of Salinity and calculation of mixing time scales. Netherlands Journal of Sea Research 10:149–191.

Chapter 2

Winter feeding activity of the common starfish

Antonio Agüera
Michel Trommelen
Frances Burrows
Tim Schellekens
Jeroen M. Jansen
Aad C. Smaal

Published in: Journal of Sea Research (2012)
72: 106 - 112
doi: 10.1016/j.seares.2012.01.006

Abstract

In the Wadden Sea the common starfish is an important predator of mussel beds, which in turn are a relevant ecological and economic resource. To improve the management of mussel seedbeds, knowledge is required on over winter predation, a factor affecting mussel survival. The aim of this study was to assess the importance of *A. rubens* feeding activity during winter and how it relates with changes in temperature. Feeding activity of starfish was monitored during a full winter. The potential impact of temperature change on starfish-mussel seed interactions during winter was analysed. The factor shading was included, as changes in light intensity appear to be a primary governing factor for the timing of feeding activity. The results showed that temperature limits feeding rate and feeding activity of starfish during winter. However, starfish feeding rate exhibited very high sensitivity to temperature changes. Light intensity affected both feeding rate and feeding activity. It is concluded that starfish may not be an important factor destabilizing seedbeds during a mean winter, but its importance may grow along with the increasing temperature due to climate change.

2.1 Introduction

Mussel beds are an important ecological component in the Wadden Sea. Being opportunistic, their offspring settles anywhere they find a suitable substrate creating new beds composed entirely by one cohort of mussels. Newly formed mussel beds function as a habitat for various invertebrates (Buschbaum et al. 2008) and benthic fishes, and as a food source for birds (Dankers and Zuidema 1995, Nehls et al. 1997, Leopold et al. 2001). Moreover, these beds are commercially important, providing seed to stock the numerous culture lots in the area.

Young mussel beds may disappear within months after formation due to factors such as environmental perturbations (Caceres-Martinez et al. 1994, Freitas et al. 2007, Jansen et al. 2009), intraspecific competition (O'Neill et al. 1983, Petraitis 1995) and predation (O'Neill et al. 1983, Navarrete and Menge 1996). In the Wadden Sea, a stability index is used to assess which sublittoral young mussel beds can be harvested by the mussel fishery; this stability index explains the probability of those beds to survive the winter and it is essential for the management of mussel seed fishing and mussel bed restoration in the Wadden Sea (Brinkman et al. 2003). To improve this index, knowledge is required on over winter predation on young mussel beds, as a factor affecting stability.

Common starfish (*Asterias rubens* L.) is a quantitatively important predator on mussels in the Wadden Sea (Saier, 2001), where it only occurs in subtidal areas. Starfish are considered ‘keystone predators’ in many ecosystems (Menge 1982, Uthicke et al. 2009). They have the capacity to control the distribution and abundance of their prey (Paine 1974, Paine et al. 1985, Gallagher et al. 2008) and are well known to occur at high concentrations on mussel beds (Dare 1982, Gallagher et al. 2008). Intense starfish predation has also been described for the Wadden Sea (Saier 2001), where extensive aggregations feed on new mussel seedbeds during summer and autumn. Feeding continues until entire beds have been eliminated (Gallagher et al. 2008), or until winter comes and temperatures become too low (Hancock 1955, Anger et al. 1977).

Like all poikilotherms, *A. rubens* exhibits a temperature dependent metabolic rate. The low temperature threshold, as well as the temperature range of these ectotherms’ metabolism will determine the faith of many young mussel beds in the Wadden Sea. The study of the seasonal variability of *A. rubens* predation rate is not new. A number of field observations and a few laboratory experiments had been undertaken (Hancock 1955, 1958, Anger et al. 1977) describing a decrease in feeding rate during winter. To that effect Castilla (1972a, 1972b) and Castilla & Crisp (1970) added studies on the seasonality in the response of *A. rubens* to prey. There are, therefore, different interpretations of this seasonal variability, suggesting that *A. rubens* feeding activity may be affected not only by temperature but by other factors as the production of attractants by the prey and the physiological state of the animal (Sloan 1980). However, it has not been assessed yet how temperature affects feeding rate and its role in the survival or extermination of young mussel beds.

Changes in light intensity appear to be a primary governing factor for the timing of feeding activity in *A. rubens* (Briggs 1983). However literature is not entirely conclusive. *A. rubens* has been variously described as a daylong forager (Eriksson et al. 1975), nocturnal predator (Ebling et al. 1966, Thain 1971) or diurnal predator with reduced levels of activity at night and peaks at dawn and dusk (Anger et al. 1977). In laboratory experiments, Castilla & Crisp (1970) found that *A. rubens* exhibited a negative reaction to light, moving away from a light source. These authors also explained that this reaction changed when they were acclimated. It can be concluded that the behaviour to light by *A. rubens* is variable, and probably depends on several environmental factors (Castilla 1971, Sloan 1980). In a complex environment like the Wadden Sea, where turbidity is very high (de Jonge et al. 1993) creating a continuous twilight, even during the brightest days, the influence of a shaded environment on *A. rubens* feeding activity may be relevant.

The aim of this study was to assess the importance of *A. rubens* feeding on mussel seedbeds during winter and to determine the effect of temperature. For that, feeding rate of *A. rubens* was followed during a full winter, including transitions from autumn and toward spring were observed. The role of temperature on feeding activity seasonal variation was studied, modelled and discussed in a climate change context. The potential impact of temperature change on starfish-mussel seed interactions during winter was analysed. Additionally an effect of light intensity on feeding rate was analysed and possible effects of temperature in *A. rubens* photophysiology disentangled.

2.2 Material & Methods

2.2.1 Sample collection and storage

A series of outdoor mesocosm experiments was carried out between October 2009 and April 2010 to calculate *A. rubens* individual feeding rate on mussel seed during winter.

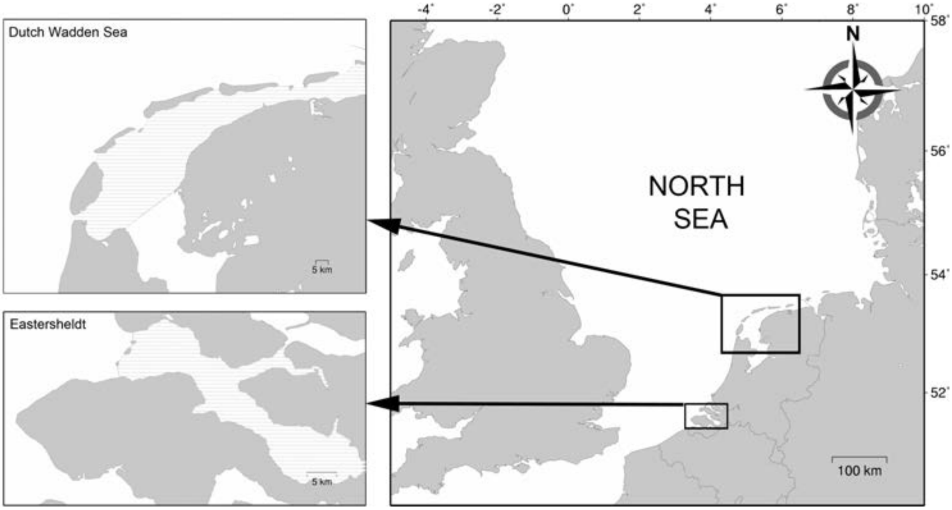


Figure 2.1. Map indicating sampling localities.

A total of 50 specimens of *A. rubens* were obtained from subtidal mussel seedbeds at the Eastern Scheldt estuary (Figure 2.1) by SCUBA diving. Starfish size ranged from 30 to 80 mm arm length (Mean= 57.7 mm; SD= 12.5 mm), measured from the centre to the tip of the largest arm to the nearest millimetre, corresponding to a mean weight of 42.97 g (SD=25.23 g). Collected specimens did not reproduce while the experiment took place, however some animals spawned shortly after the last experiment was finished. This experimental starfish stock was distributed over 6 tanks that received a continuous supply of water from the Eastern Scheldt, with a stable salinity of 30 PSU. Starfish used in the experiments were not labelled or identified in any way. Experimental tanks were covered, creating a continuously shaded environment. Water natural thermal fluctuations in the tanks were monitored. Stocked starfish were fed “add libitum” with live mussel seeds and broken mussels. Before the first experiment took place and once again after the coldest winter weeks, the full starfish stock was measured and weighed to calculate wet weight-arm length correlations.

Seed mussels were also obtained from the Eastern Scheldt. These were collected using a small mussel dredge. A second batch of mussel seeds was obtained from the Wadden Sea and mixed with those from the Eastern Scheldt. Mussels ranged from 15 to 33 mm shell length (Mean=22.8 mm; SD= 2.9 mm), measured to the nearest tenth of mm with soft tissue weighting a mean of 1.10 g (SD=0.64 g). Mussels were not yet mature and did not reproduce during the experiments. Seeds were stored in a 4000l tank with continuous water flow-through from the Eastern Scheldt.

Starfish and mussel seed were selected randomly to obtain a distribution representative of the actual population present in the Wadden Sea and Eastern Scheldt at the moment of sampling.

Both, mussels and starfish were kept in their storage tanks 3 weeks prior to the start of the experiments allowing the animals to acclimate to captivity conditions.

2.2.2 Experimental set-up

The experimental set-up involved two blocks consisting of 15 tanks of 15l each. All tanks had sand as substrate. Water from the Eastern Scheldt was continuously supplied to all tanks, ensuring that the specimens under observation were exposed to water natural thermal variation and oxygenated conditions. Water temperature in the experimental tanks was monitored using HOBO® temperature loggers, distributed between both setups, recording water temperature every 15 minutes.

Experiments were carried out under natural photoperiod conditions. However, one of the two setups was covered with a tarpaulin cloth that attenuated 99% of light intensity during experiments; the other setup was covered by a net to avoid bird predation.

Twelve consecutive experiments (run in both setups simultaneously) were undertaken, each one lasting between 1 to 2 weeks. In each experiment, clumps of 20 – 30 mussel seeds were placed in 25 of the 30 tanks (one clump per tank), 11 in shaded tanks, another 11 in un-covered tanks and the other three changing from experiment to experiment but with at least one at each set-up. The tanks holding the mussel clumps were selected randomly by random number generation. Subsequently, 22 (11 un-covered and 11 shaded) of these tanks received a starfish; those were roughly selected by eye from the stock tanks with the aim of using a similar length distribution in all the experiments. The experiment started at the time starfish were introduced in the tanks. The last three tanks were left without starfish and used as controls for mussel mortality. Starfish arm length was measured at the start of the experiment. Each experiment was run until starfish had eaten approximately half of the clump. If starfish did not eat the experiment was ceased after two weeks. At the end of each experiment the number of consumed seeds in each tank was counted.

2.2.3 Data Analysis

Feeding rate of *Asterias spp.* has been generally presented as a function of starfish size (arm length or diameter) (Hancock 1955, 1958, Dolmer 1998, Saier 2001). In the population sampled for the present experiment, 99% of starfish wet weight variability was explained by a power function of arm-length. Moreover starfish feeding rate presented a strong co-linearity with starfish wet weight (p-value <0.001, $R^2 = 0.256$). Therefore wet weight was chosen to standardise feeding rate by starfish size as the number of mussels consumed per hour and per gram of starfish (wet weight) eliminating the effect of starfish size.

The mean temperature of each experiment was calculated from the temperature loggers' output.

A zero-inflated negative binomial model (ZINB) (Zuur et al. 2009) was used to analyse the effect of temperature and the shading treatment in feeding rate and feeding activity for the zero inflated over-dispersed dataset (Figure 2.2). This approach is able to deal with an over dispersed dataset due to zero inflation. This model consists of two outputs: a count model and a zero-inflation model (Table 2.1). The zero inflation part models the probability of counting zeros and uses covariates to predict for the excess of zeros, the count part models the observed variable in function of predictors taking into account the excess of zeros that are calculated by the former.

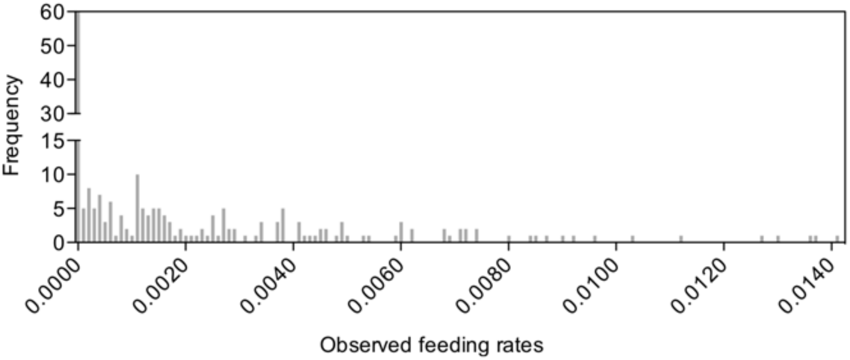


Figure 2.2 Frequency distribution of observed feeding rates during the experiment. Feeding rate of *Asterias rubens* as number of mussels · hour^{-1} · g^{-1} (wet weight *A. rubens*).

A first model was fitted to test the effect of temperature and shading on feeding rate and the effect of temperature, shading and the duration of each experiment in the probability of recording a zero. A final model was deducted from the interactions encountered in this first model in order to obtain the best fit for the observed data (Zuur et al. 2009). Therefore a series of models dropping the variables that resulted non-significant in that first approach were conducted. The resulting models were compared by means of Akaike Information Criterion (AIC) and Likelihood ratio test, and the model formulation that fitted the data best was used to explain the results of this study. To validate the final model, plots of Pearson residuals against fitted values and each of the explanatory variables were performed and are available as online resource.

In order to assess the sensitivity of *A. rubens* feeding rate to changes in temperature, different Arrhenius temperatures (T_A) were calculated by the linear regression: $Ln(feeding\ rate) = a + T_A \bullet (1/K)$; where K is the temperature in Kelvins. Arrhenius should be calculated in the range where the measured rate is positive; therefore model fitted values were used instead of observed to avoid zeros. However, due to differences caused by the shading treatment in feeding rate, model predicted values for shaded and un-covered values within the same temperature range were used to calculate two alternative T_A.

All the statistical analyses were performed using R v.12.2. (http://www.r-project.org/) and count data regression models from *pscl* package (Zeileis et al. 2008).

2.3 Results

A. rubens feeding rate ranged from 0 to 0.014 mussels · hour^{-1} · g^{-1}. Highest feeding rate was observed in April and November at a water temperature around 10 °C. Towards lower temperatures, feeding rate decreased rapidly, and was found to be close to zero or zero between 0 and 3°C. Low mean feeding rate values during winter resulted partly from starfish that were not feeding during the experiments. At 0º C about 70% of the individuals did not consume any mussel during two weeks, with none feeding in the un-covered setup. The highest feeding rate during the colder winter months (0 – 2 ºC) was 0.0014 mussels · hour^{-1} · g^{-1} (recorded under shaded conditions). That resulted in a seasonal variability in *A. rubens* feeding rate (Figure 2.3). No temperature differences were recorded between the shaded and un-covered setups. No mussel mortality was recorded at the controls in any of the experiments.

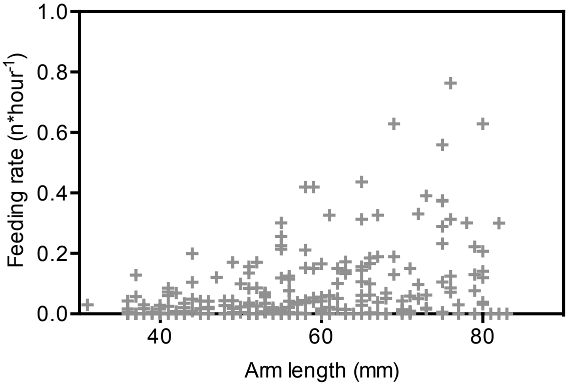


Figure 2.3 Feeding rate at size. Feeding rate as number of mussels · hour^{-1}

Low feeding rate was observed over the full starfish size-range (Figure 2.4). The relation between feeding rate and starfish size resulted in a typical triangular scatterplot, with the upper edge of the data points revealing the potential feeding rate as a function of starfish size.

The first multiregression analysis showed that only temperature had an important effect on the observed feeding rate (*z*=2.34, *p-value*=0.019). In addition to temperature, shading had a significant effect on the high number of non-feeding individuals recorded (*z*=-3.72, *p-value*<0.001) (Figure 5B).

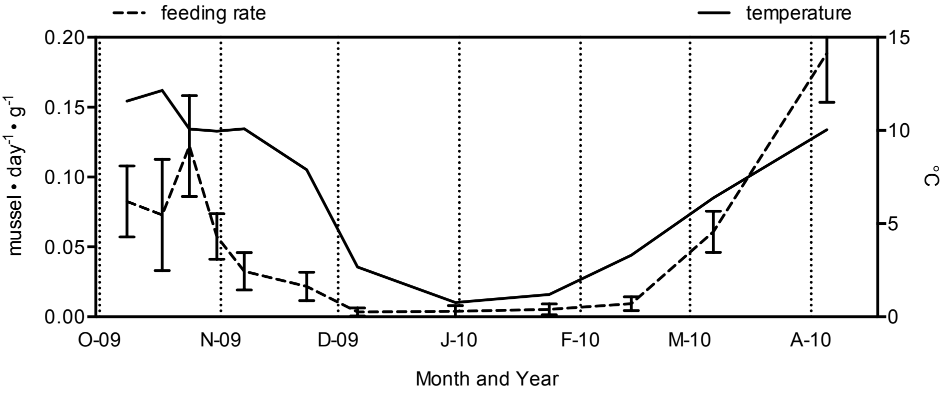


Figure 2.4 Observed feeding rates by date and temperature between October 2009 and April 2010. Feeding rate as mussels · day^{-1} · g^{-1} (wet weight *A. rubens*)

Table 2.1. Formulation and estimated coefficients of the multiregression analysis. In the formulation *fr* is feeding rate (counts: mussels · hour^{-1} · g^{-1}); *p* is the probability of a zero count.

$$fr = e^{a+b_1 \cdot T+b_2 \cdot S}$$

Count Model coefficients (negative binomial)

	Estimate	Std. Error	z	p-value
Intercept	(*a*) 0.35343	0.32251	1.096	0.2731
Temperature (*T*)	(b_1) 0.25472	0.02263	11.254	<0.001
Shading treatment (*S*)	(b_2) 0.51353	0.13534	3.794	<0.001

$$p = \frac{e^{v+c_1 \cdot T+c_2 \cdot S+c_3 \cdot T \cdot S}}{1+e^{v+c_1 \cdot T+c_2 \cdot S+c_3 \cdot T \cdot S}}$$

Zero-inflation model coefficients (binomial)

	Estimate	Std. Error	z	p-value
Intercept	(*v*) 6.5084	1.517	4.29	<0.001
Temperature (*T*)	(c_1) -1.2213	0.3177	-3.844	<0.001
Shading treatment (*S*)	(c_2) -3.636	0.8772	-4.145	<0.001
Temperature · Shading	(c_3) 0.5534	0.1704	3.248	0.0016

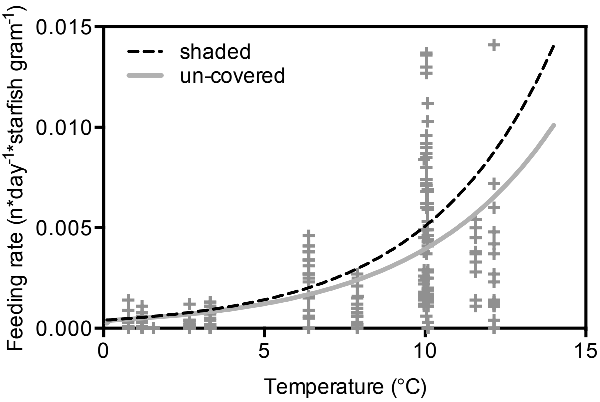


Figure 2.5 Feeding rate as a function of temperature. Feeding rate as mussels · hour^{-1} · g^{-1} (wet weight of *A. rubens*). Lines represent the ZINB model output.

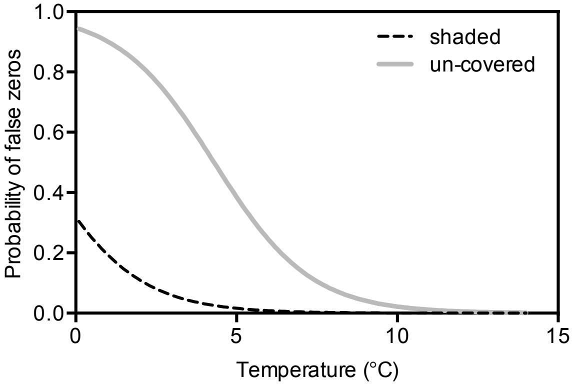


Figure 2.6 Probability of sampling a non-feeding *A. rubens* as a function of temperature.

This model provided better fit (compared by AIC) and simpler output (5 to 7 covariates) than the best fit when compared to another model performed using feeding rate that were not standardised by specimen wet weight.

Arrhenius plots revealed a high sensitivity of feeding rate to temperature (Figure 2.7), meaning that *A. rubens* feeding rate increased or decreased rapidly with small changes in temperature. There is a significant difference between the calculated T_A for un-covered and shaded treatments ($F = 277.91$; p-value < 0.0001).

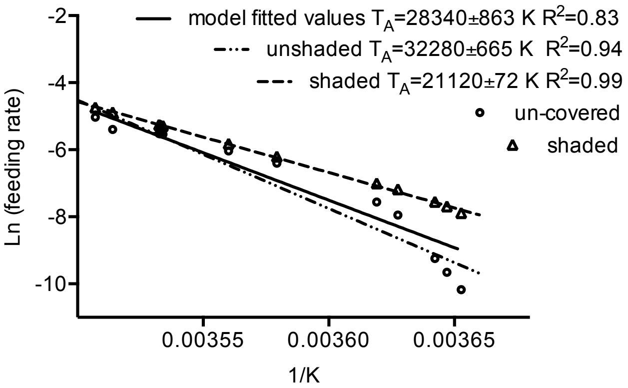


Figure 2.7 Arrhenius plots for feeding rates. Feeding as mussels · hour^{-1} · g^{-1} (wet weight *A. rubens*). T_A, Arrhenius temperature (± SD).

2.4 Discussion

The aim of this study was to assess *A. rubens* predation impact on mussel seedbeds survival from autumn to winter and from winter to spring. As expected, our results showed a reduction of feeding activity during winter. This has been recorded in previous studies (Hancock 1955, 1958, Briggs 1983) where feeding rate on various food items were studied. Other studies measured a reduction of growth during winter (Barnes and Powell 1951, Jangoux and Impe 1977, Barker and Nichols 1983, Nichols and Barker 1984) generally linked to a decrease in temperature and food availability.

The zero-inflated over-dispersed dataset (Figure 2.2) of feeding rate resultant from this study can be explained by the bulk feeding behaviour of *A. rubens* (Sloan 1980). Starfish may consume large quantities during a short time period and digests, without further feeding, during several days. As a result of this behaviour, especially at lower temperatures, non-feeding individuals were often observed. Shading treatment reduced the number of non-feeding individuals.

Although this study was carried out at the Eastern Scheldt, its results are easily extensible to all Dutch estuaries, including the Wadden Sea. Intensive mussel culture in the Eastern Scheldt involves the transportation of high number of seeds and several young starfish annually from the Wadden Sea allowing high degree of mixing between Wadden Sea and Eastern Scheldt mussel populations. Also water temperature in Dutch estuaries (including the Eastern Scheldt) and the Wadden Sea are linked, following a similar seasonal pattern (van Aken 2010).

2.4.1 Temperature

When analysing the observed feeding rate, it is clear that temperature explained most of the variation (Table 2.1; Figure 2.5). Metabolic rates, feeding rate and growth rates of *A. rubens* are limited when temperature decreases during the winter season, as has been already observed for *A. rubens* (Hancock 1955, 1958) and other asteroids (Watts and Lawrence 1986, 1990, Sanford 2002). They all agreed on the importance of temperature on the feeding rate of starfish.

It could be concluded that *A. rubens* do feed during winter, but predation decreases at low temperatures and most of them appear to stop feeding when temperatures reach 2 ºC or less, as was also observed by Hancock (1958). Although feeding rate at temperatures between 0 and 4 °C were close to zero, some feeding was still observed (particularly in the shaded treatment), suggesting that these low water temperatures were not below a metabolic temperature threshold, and did not result in a stage of dormancy.

Field observations and laboratory experiments made by Hancock (1955) suggested that the optimal feeding temperature for starfish lies between 10 and 13ºC. In the present study the highest feeding rate was observed at 10ºC. The steep increase of feeding rate with temperature in spring could suggest that the optimum may not be near yet. That fast increase may be also explained otherwise; as starfish used in this experiments started to spawn shortly after the last experiment was finished. The increasing rate may have been due to the necessity of *A. rubens* to build up energy for reproduction, specially after a harsh winter. It is known that *A. rubens* accumulate energy until just before reproduction and that those reserves determine the fecundity of the animals (Jangoux and Impe 1977). However, *A rubens* stop feeding before spawning and during it (Sloan 1980), and that may produce a decrease in the steep increase of feeding rate observed in spring in this experiment. Additional measurements under summer conditions and different physiological stages could improve our understanding of the optimum feeding temperature for the starfish in Dutch estuarine areas.

Arrhenius temperatures calculated here showed an increase of feeding rate with temperature that is much higher than those previously observed in other asteroids (Barbeau and Scheibling 1994). The lowest T_A obtained of 20970K (for 1-14 ºC) for shaded starfish is comparable to the T_A = 16015K (4 –15 ºC) calculated from Barbeau & Scheibling (1994) for the closely related species *Asterias vulgaris.* The difference could probably be explained by the larger temperature range in this study. The thermal sensitivity of the feeding rate of *A. rubens* is much higher than that calculated for any other mussel predator present in the Wadden Sea (Freitas et al. 2007). That suggested that *A. rubens* feeding rate could be drastically affected by the changes of water temperature in a global climate change context.

2.4.2 Shading

The results showed an effect of shading on feeding rate and feeding activity. Reduced light intensity increases both the number of *A. rubens* recorded feeding and the feeding rate. Changes in light intensity appeared to be a primary governing factor for the timing of feeding activity in this experiment, however habitat and behavioural differences may mediate the response. This study results agree with a preference for feeding in the dark or twilight that have been previously described by Briggs (1983), a behaviour that may have evolved from the avoidance of possible predators.

An interaction between shading treatment and temperature was found in these experiments. This is only significant in the number of non-feeding individuals recorded during each experiment. At low temperatures the animals in the shade did not stop feeding while most of them stopped in the un-covered set-up. That suggests that *A. rubens* still need to feed at low temperature. In other Asteroids it had been suggested that harsh temperatures that may limit movements but not the necessity of feeding (Watts and Lawrence 1990). Therefore it can be thought that the shading treatment provided a “safer” environment and encouraged feeding.

A. rubens stock used in the experiments was kept in shaded tanks until they were used in the experiments. *A. rubens* is indifferent to light when acclimated to dark (Castilla and Crisp 1970, Castilla 1971), however, it cannot be ruled out that part of the differences in shaded/un-covered experiments may be caused by acclimation to shaded conditions. Castilla (1971) also stated that at low temperatures starfish are indifferent to light, a fact that our experiments contradict with a higher effect of the shading treatment with the decrease of temperatures.

The shaded treatment is relevant in the case of the Wadden Sea where turbidity is very high. Previous results of other studies based of observations on clear waters or a 12/12 photoperiod laboratory conditions may underestimate the predation capacity of *A. rubens* when applied to the Wadden Sea.

2.4.3 Ecological implications

High densities of *A. rubens* have been reported several times (Brun 1968, Nauen 1978, Sloan and Aldridge 1981, Dare 1982, Guillou 1996), they seem to be related to abundance of food and encouraged by favourable environmental conditions. Densities are also much higher when starfish are forming feeding fronts, where densities over 12 kg/m^2 of *A. rubens* have been recorded (Dare 1982). In the Wadden Sea densities up to 600 g/m^2 have been reported (Saier 2001).

At higher water temperature, feeding rate reached 0.34 mussels $\cdot$ day^{-1} $\cdot$ g^{-1} of starfish wet weight. If an mean starfish density of 300 g m^{-2} is considered it will take less than 10 days to eliminate the seedbed entirely, knowing that the mean density in a mussel seedbed formed by mussel seeds of 15 to 33 mm shell length is often found to be to be around 1000 individuals m^{-2} (Figure 2.8). This value could be thought to be conservative as densities over 500 starfish m^{-2} are commonly found in the Wadden Sea (van Stralen et al. 2005) and that *A. rubens* as small as 10 mm arm length are already able to consume mussel seeds over 10 mm shell length (O'Neill et al. 1983). Van Stralen et al. (2005) also report densities between 1000 and 3000 individuals/m^2 for mussel seeds between 15 – 30 mm.

In view of the results *A. rubens* predation effects on winter seedbed stability depends greatly of winter temperature. During most winters, the potential impact of starfish on seedbeds will be negligible. However, it is important to take into account that during 2009 – 2010 winter registered temperatures were considerably below the Wadden Sea mean for the last decade (van Aken 2008).

In a global warming scenario as that used by Brinkman et al. (2001) with an increase of temperature by 2° C by 2050 and 4° C by 2100. In that context the time needed by *A. rubens* to eliminate a mussel bed will be greatly reduced as feeding rate of *A.*

rubens on mussels will double with an increase of 2º C and multiplied by four with an increase of 4° C.

In estuarine areas like the Wadden Sea, the impact of climate change will be partly determined by how the size-selective interactions are affected by temperature (Freitas et al. 2007). At low temperatures starfish growth is reduced and even some shrinking may occur (Jangoux and Impe 1977, Guillou et al. 2012). However, although it depends of many factors, mussel shell grows during winter (De Mesel et al. 2009). It is possible then that many mussels may outgrow *A. rubens* during winter and attain size refuge, as the maximum mussel size *A. rubens* are able to prey on is determined by their own size (O'Neill et al. 1983, Dolmer 1998, Hummel et al. 2011). A similar effect has been observed in scallop culture plots, where seeding during winter improved seed survival as scallops have time to grow before starfish become active (Barbeau and Scheibling 1994). Although mussel seeds will probably grow faster at higher temperatures (De Mesel et al. 2009), the high sensitivity of starfish feeding rate to temperature suggests that they may not grow fast enough to outgrow starfish before the seedbed is depleted. Therefore, an increase in winter temperature may lead to a decrease in the seedbed winter stability.

It is important to point out, that although we have seen the evolution of *A. rubens* during winter and that this is highly correlated with temperature, the effect of temperature raising during winter have not been tested in this study.

In conclusion, *A. rubens* predation is reduced during winter. *A. rubens* feeding rate exhibits a high sensitivity to temperature, consequently, small changes in environmental temperature produce significant reactions in starfish predation. *A. rubens* may not be an important factor destabilizing seedbeds during winter nowadays, but its importance may grow along with the increasing temperature due to climate change.

References

Anger, K., U. Rogal, G. Schriever, and C. Valentin. 1977. In-situ investigations on the echinoderm *Asterias rubens* as a predator of soft-bottom communities in the western Baltic Sea. Helgoländer Wissenschaftliche Meeresuntersuchungen 29:439–459.

Barbeau, M. A., and R. E. Scheibling. 1994. Behavioral mechanisms of prey size selection by sea stars (*Asterias vulgaris* Verrill) and crabs (*Cancer irroratus* Say) preying on juvenile sea scallops (*Placopecten magellanicus* (Gmelin)). The Journal of Experimental Marine Biology and Ecology 180:103–136.

Barker, M. F., and D. Nichols. 1983. Reproduction, Recruitment and juvenile ecology of the starfish, *Asterias rubens* and *Mathasterias glacialis*. Journal Of The Marine Biological Association Of The United Kingdom 63:745–765.

Barnes, H., and H. T. Powell. 1951. The growth-rate of juvenile *Asterias rubens* L. Journal Of The Marine Biological Association Of The United Kingdom 30:381–381.

Briggs, C. F. 1983, February. A study of some sublittoral populations of Asterias rubens (L.) and their prey. Port Erin.

Brinkman, A. G., B. J. Ens, K. Kersting, M. Baptist, M. Vonk, J. Drent, B. M. Janssen-Stelder, and M. W. M. van der Tol. 2001. Modelling the impact of climate change on the Wadden Sea ecosystems. Dutch National Research Programme on Global Air Pollution and Climate Change. 410200066.

Brinkman, A. G., T. Bult, N. Dankers, A. Meijboom, M. van Stralen, and J. de Vlas. 2003. Mosselbanken : Kenmerken , Oppervlaktebepaling en Beoordeling van Stabiliteit. Wageningen Alterra. Report 707. Pages 70.

Brun, E. 1968. Extreme population density of the starfish *Asterias rubens* on a bed of Iceland scallops. Astarte 32:1–3.

Buschbaum, C., S. Dittmann, J.-S. Hong, I.-S. Hwang, M. Strasser, M. Thiel, N. Valdivia, S.-P. Yoon, and K. Reise. 2008. Mytilid mussels: global habitat engineers in coastal sediments. Helgoland Marine Research 63:47–58.

Caceres-Martinez, J., J. Robledo, and A. Figueras. 1994. Settlement and post-larvae behaviour of *Mytitus galloprovincialis*: field and laboratory experiments. Marine Ecology Progress Series 112:107–117.

Castilla, J. C. 1971. Responses to light in *Asterias rubens*. Pages 495–511 *in* D. J. Crisp, editor. Cambridge.

Castilla, J. C. 1972a. Responses of *Asterias rubens* to bivalve prey in a Y-maze. Marine Biology 12:222–228.

Castilla, J. C. 1972b. Avoidance behaviour of *Asterias rubens* to extracts of *Mytilus edulis*, solutions of bacteriological peptone, and selected amino acids. Marine Biology 15:236–245.

Castilla, J. C., and D. J. Crisp. 1970. Responses of *Asterias rubens* to olfactory stimuli. Journal Of The Marine Biological Association Of The United Kingdom 50:829–847.

Dankers, N., and D. R. Zuidema. 1995. The Role of the Mussel (*Mytilus edulis* L.) and Mussel Culture in the Dutch Wadden Sea. Estuaries 18:71–71.

Dare, P. J. 1982. Notes on the swarming behaviour and population density of *Asterias rubens* L. (Echinodermata: Asteroidea) feeding on the mussel, *Mytilus edulis* L. Journal du Conseil 40:112–118.

de Jonge, V. N., K. Essink, and R. Boddeke. 1993. The Dutch Wadden Sea : a changed ecosystem. Hydrobiologia:45–71.

De Mesel, I., J. W. M. Wijsman, and A. van Gool. 2009. Groeimetingen op percelen in de westelijke Waddenzee. Page 21. Wageningen IMARES.

Dolmer, P. 1998. The interactions between bed structure of *Mytilus edulis* L. and the predator *Asterias rubens* L. The Journal of Experimental Marine Biology and Ecology 228:137–150.

Ebling, F. J., A. D. Hawkins, J. A. Kitching, L. Muntz, and V. M. Pratt. 1966. The Ecology of Lough Ine XVI. Predation and Diurnal Migration in the Paracentrotus Community. Journal of Animal Ecology 35:559–566.

Eriksson, S., S. Evans, and B. Tallmark. 1975. On the coexistence of scavengers on shallow, sandy bottoms in Gullmar fjord (Sweden). Zoon 3:65–70.

Freitas, V., J. Campos, M. Fonds, and H. W. Van der Veer. 2007. Potential impact of temperature change on epibenthic predator–bivalve prey interactions in temperate estuaries.

Gallagher, T., C. A. Richardson, R. Seed, and T. Jones. 2008. The Seasonal movement and abundance of the starfish *Asterias rubens* in relation to mussel farming practive: a case study from the Menai Strait, UK. Journal of Shellfish Research 27:1209–1215.

Guillou, M. 1996. Biotic and abiotic interactions controlling starfish outbreaks in the Bay of Douarnenez, Brittany, France. Oceanologica Acta 19:415–420.

Guillou, M., G. Joly-Turquin, S. Leyzour, P. Pernet, and P. Dubois. 2012. Factors controlling juvenile growth and population structure of the starfish Asterias rubens in intertidal habitats: field and experimental approaches. Journal Of The Marine Biological Association Of The United Kingdom 92:367–378.

Hancock, D. A. 1955. The Feeding Behaviour of Starfish on Essex Oyster beds. Journal Of The Marine Biological Association Of The United Kingdom 34:313–331.

Hancock, D. A. 1958. Notes on Starfish on an Essex Oyster bed. Journal Of The Marine Biological Association Of The United Kingdom 37:565–589.

Hummel, C., P. Honkoop, and J. van der Meer. 2011. Small is profitable: No support for the optimal foraging theory in sea stars *Asterias rubens* foraging on the blue edible mussel *Mytilus edulis*. Estuarine, Coastal and Shelf Science 94:89–92.

Jangoux, M., and E. V. Impe. 1977. The annual pyloric cycle of *Asterias rubens* L. (ECHINODERMATA: ASTEROIDEA). The Journal of Experimental Marine Biology and Ecology 30:165–184.

Jansen, J. M., H. Hummel, and S. W. Bonga. 2009. The respiratory capacity of marine mussels (Mytilus galloprovincialis) in relation to the high temperature threshold. Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 153:399–402.

Leopold, M. F., R. K. H. Kats, and B. J. Ens. 2001. Diet (preferences) of Common Eiders *Somateria mollissima*. Wadden Sea Newsletter:25–31.

Menge, B. A. 1982. Effects of feeding on the environment: Asteroidea. Pages 521–551 *in* M. Jangoux, J. M. Lawrence, and J. M. Lawrence, editors. Echinoderm Nutrition. A.A. Balkema, Rotterdam.

Nauen, C. E. 1978. The growth of the sea star, *Asterias rubens*, and its role as benthic predator in Kiel Bay. Kieler Meeresforschungen 4:68–81.

Navarrete, S., and B. A. Menge. 1996. Keystone Predation and Interaction Strength: Interactive Effects of Predators on Their Main Prey. Ecological Monographs 66:409–429.

Nehls, G., I. Hertzler, and G. Scheiffarth. 1997. Stable mussel *Mytilus edulis* beds in the Wadden - They're just for the birds. Helgoländer Meeresuntersuchungen 51:361–372.

Nichols, D., and M. F. Barker. 1984. Growth of juvenile *Asterias rubens* L.(ECHINODERMATA:ASTEROIDEA) on an intertidal reef in southwestern Britain. The Journal of Experimental Marine Biology and Ecology 78:157–165.

O'Neill, S., A. Sutterlin, and D. Aggett. 1983. The effects of size-selective feeding by starfish (*Asterias vulgaris*) on the production of mussels (*Mytilus edulis*) cultured on nets. Aquaculture 35:211–220.

Paine, R. T. 1974. Intertidal community structure. Oecologia 15:93–120.

Paine, R. T., J. C. Castilla, and J. Cancino. 1985. Perturbation and Recovery Patterns of Starfish-Dominated Intertidal Assemblages in Chile, New Zealand, and Washington state. The American Naturalist 125:679–691.

Petraitis, P. S. 1995. The Role of Growth in Maintaining Spatial Dominance by Mussels (*Mytilus edulis*). Ecology 76:1337–1346.

Saier, B. 2001. Direct and indirect effects of seastars *Asterias rubens* on mussel beds (*Mytilus edulis*) in the Wadden Sea. Journal of Sea Research 46:29–42.

Sanford, E. 2002. The feeding, growth, and energetics of two rocky intertidal predators (*Pisaster ochraceus* and *Nucella canaliculata*) under water temperatures simulating episodic upwelling. The Journal of Experimental Marine Biology and Ecology 273:199–218.

Sloan, N. A. 1980. Aspects Of The Feeding Biology. Oceanography and Marine Biology: an Annual Review 18:57–124.

Sloan, N. A., and T. H. Aldridge. 1981. Observations on an aggregation of the starfish *Asterias rubens* L. in Morecambe Bay, Lancashire, England. Journal of Natural History 15:407–418.

Thain, V. M. 1971. Diurnal rhythms in snails and starfish. Pages 513–537 *in* D. J. Crisp, editor. 4th European marine biology symposium. Cambridge University Press. Cambridge.

Uthicke, S., B. Schaffelke, and M. Byrne. 2009. A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. Ecological Monographs 79:3–24.

van Aken, H. M. 2008. Variability of the water temperature in the western Wadden Sea on tidal to centennial time scales. Journal of Sea Research 60:227–234.

van Aken, H. M. 2010. Meteorological forcing of long-term temperature variations of the Dutch coastal waters. Journal of Sea Research 63:143–151.

van Stralen, M., J. Craeymeersch, and J. Jol. 2005. Inventarisatie van het wilde mosselbestand in de Waddenzee in het najaar van 2005. Report 2005.48.2. Pages 19.

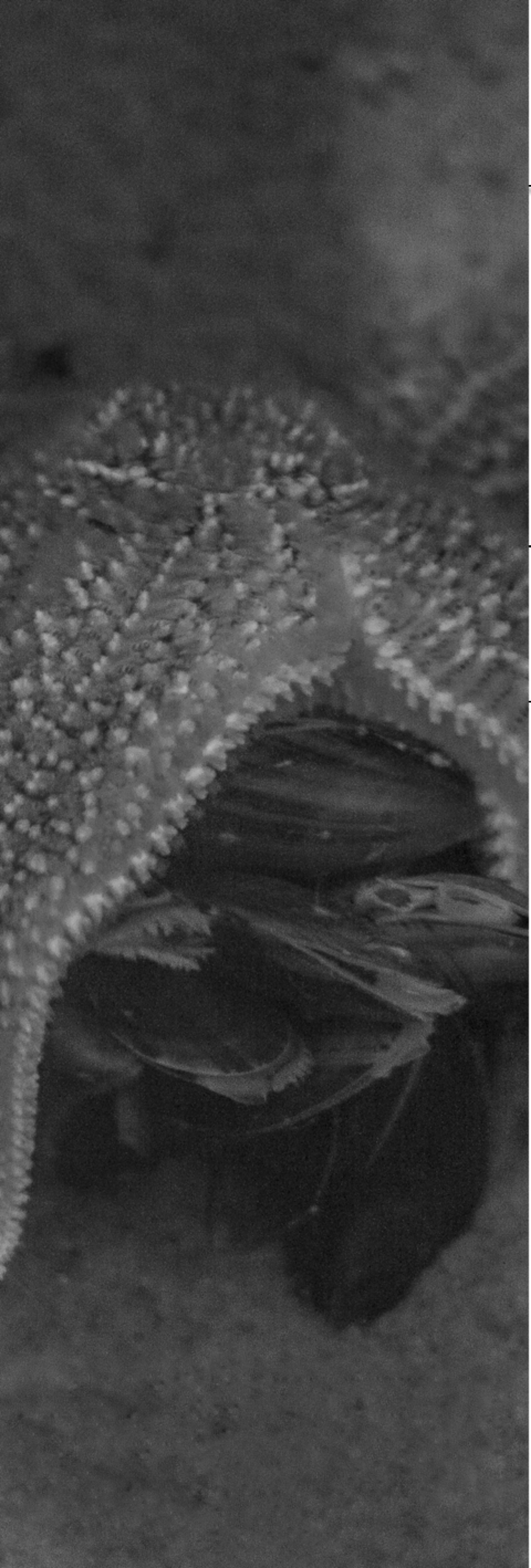
Watts, S., and J. M. Lawrence. 1986. Seasonal effects of temperature and salinity on the organismal activity of the seastar *Luidia clathrata* (say) (Echinodermata: Asteroidea). Marine and Freshwater Behaviour and Physiology 12:161–169.

Watts, S., and J. M. Lawrence. 1990. The effect of temperature and salinity interactions on righting, feeding and growth in the sea star *Luidia clathrata* (Say). Marine and Freshwater Behaviour and

Physiology 17:159–165.

Zeileis, A., C. Kleiber, and S. Jackman. 2008. Regression Models for Count Data in R. Journal Of Statistical Software 27.

Zuur, A. F., E. N. Ieno, A. A. Savelier, and G. M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R (statistics for Biology and Health). Pages 574–574. Springer, Berlin.



Chapter 3

Effects of osmotic stress on the predation behaviour of starfish

Antonio Agüera
Tim Schellekens
Jeroen M. Jansen
Aad C. Smaal

Accepted for publication:
Journal of Sea Research

Abstract

Environmental stress plays an important role in determining ecosystem functioning and structure. In estuarine areas both tidal and seasonal salinity changes cause osmotic stress on predators affecting their behaviour and survival. The effect on performance is reflected in the interaction between these predators and their prey, determining the predator impact on the prey population. The common starfish, *Asterias rubens* inhabits estuarine areas, such as the Dutch Wadden Sea, that exhibit large seasonal variation in salinity (10 – 32 PSU). In those areas *A. rubens* is an important shellfish predator that exerts top down control on its prey. This results in an impact on cultured and natural shellfish populations. However, the impact of *A. rubens* on its prey will be influenced by the effect of osmotic stress on its performance. Although the effect of salinity in *A. rubens* survival has been extensively studied, the impact on its predation behaviour and acclimation capacity remains unclear. In this study the performance of *A. rubens* preying on mussels (*Mytilus edulis*) after a salinity decrease is analysed and its acclimation is monitored over a period of 22 days. Results show that salinity affected performance by reducing feeding activity and causing changes in size prey selection. Moreover, as acclimation occurred, *A. rubens* predation performance improved in all, except, lethal treatments. We concluded that osmotic stress due to a salinity decrease determines *A. rubens* distribution, abundance and potential impact on the prey population. However this effect is affected by the magnitude of the change in salinity (from 31 to a minimum of 10 PSU) and its timescale (3 weeks).

3.1 Introduction

Environmental stress models have long been used to explain community structure and function (Menge and Sutherland 1976, Bertness and Grosholz 1985, Menge and Sutherland 1987, Bruno 2001). Environmental stress plays a role on community structure by affecting, among others, consumers/predators (Burnaford 2004, Robles et al. 2009, Wing and Leichter 2011). Predators are usually more sensitive to environmental stress than their prey (Menge and Sutherland 1976). That is especially the case for mobile predators of sessile prey (Menge and Sutherland 1976), as such prey are likely to be better adapted to the same stressors, due to their inability to escape stress by changing location (Saranchova 2001, Strom et al. 2012). As a result environmental stress may reduce predator activity, affecting predation performance, abundance and distribution. Moreover, depending on stress level, prey may attain different levels of refuge (Menge and Sutherland 1976). In the most extreme cases, environmental stress will cause mortality and completely exclude the predator. At lower stress levels the predator may still persist but predation will be physically and/or physiologically restricted (Menge 1978, Robles et al. 2009).

In subtidal estuarine areas salinity may exhibit large fluctuations both seasonally and tidally, thus being an important environmental factor causing osmotic stress. Predators under osmotic stress may exhibit a reduction in predation rates, growth (Stickle et al. 1985, Forcucci and Lawrence 1986) and changes in predation behaviour such as prey selection (Aronhime 2010). The final outcome of osmotic stress on the predator performance, hence its potential impact on the prey population, will be a result of the capacity of the predator to reduce the immediate effects and recover from osmotic stress (Stickle et al. 1985).

Asterias rubens is a predator that occurs often in high numbers in natural and cultured shellfish beds (Hancock 1955, Wolff 1968, Nauen 1978, Barbeau and Scheibling 1994). *A. rubens* has the capacity to exert top-down control on their prey, determining prey distribution and abundance (Nauen 1978, Menge 1982, Witman et al. 2003) and is an important factor affecting productivity in both shellfish culture (Magnesen and Redmond 2011, Agüera et al. 2012), and natural shellfish stocks (Hart 2006). *A. rubens* can be found in habitats with large salinity range (8 to 40 PSU) thus, able to exploit brackish environments such as fjords (Brun 1968), estuaries (Wolff 1968) and other locations where salinity undergoes large seasonal variations. However, despite *A. rubens* individuals occurring across a large range of salinities, it is a species with little or no capacity for osmoregulation (Binyon 1962, Shumway 1977). *A. rubens* survival under osmotic stress has been extensively studied (Kowalski 1955, Binyon 1961, Shumway 1977). Large variation was found in the minimal sustainable salinity of *A. rubens* (between 12 and 20 PSU) (Binyon 1962, Shumway 1977, Saranchova 2001). Such variation in laboratory experiments and the existence of viable populations at salinities as low as 8 PSU (Kowalski 1955) were partly explained as local adaptation (fixation) (Saranchova and Flyachinskaya 2001). However those experimental studies demonstrated that *A. rubens* will not survive the osmotic stress caused by an immediate change of 8 to 10 PSU from an acclimated salinity of 26 to 34 PSU (Binyon 1961, Shumway 1977, Saranchova 2001). However, this limit is extended when the salinity change is not immediate but rather made stepwise with fluctuation (Shumway 1977).

The Dutch Wadden Sea undergoes large seasonal variation (10 – 32 PSU) in salinity resulting from large freshwater input (anthropologically controlled) during spring melt or rainy seasons (Zimmerman 1976). Mussel beds in the area constitute an important ecological and economic resource and are impacted by *A. rubens* populations inhabiting the area. As environmental stress models hypothesize, non-lethal osmotic stress levels for *A. rubens* may still affect its performance and therefore its role as a predator. Even though limited performance may improve over time as *A. rubens* acclimates to new conditions, scenarios may arise where prey species exploit this reduced predation pressure attaining new levels of temporal or permanent refuge. Some works have previously remarked on different population dynamics among starfish populations inhabiting areas with different salinity conditions (Kowalski 1955, Guillou et al. 2012) However, the effect of non-lethal salinity changes on predation performance and the extent to which acclimation occurs in the same location following salinity changes remains unknown.

The aim of the present study was to elucidate how variation in salinity may impact predation performance of *A. rubens* when feeding on mussels. We tested the hypothesis that *A. rubens* can recover from osmotic stress caused by salinity decrease, acclimating to the new environment over time. This work focused on the acclimation process as a function of feeding activity over time, comparing among different levels of salinity decrease. Possible effects of local salinity conditions on the performance and acclimation capacity of *A. rubens* were also tested. Consequences for the cultured and wild mussel populations in both the study areas and other areas were mussels have a similar ecological and economic value are discussed.

3.2 Material & Methods

A mesocosm experiment was designed to assess the effects of decreased salinity on activity, feeding behaviour and prey size selection of *Asterias rubens*. Additionally, acclimation during the first three weeks after a salinity decrease was assessed. The study also included the effect of local adaptation to salinity regimes. To do so, in this experiment *A. rubens* individuals from two different localities exhibiting different salinity regimes were exposed to stepwise lowered salinities after initial acclimation to constant 31 PSU salinity.

3.2.1 Sample collection and storage

Asterias rubens were collected by hand from subtidal populations at the Wadden Sea and the Oosterschelde estuary (The Netherlands) (Table 3.1). The Wadden Sea exhibits a large salinity range (10 – 32.5 PSU), with salinity fluctuating both tidally and seasonally (Zimmerman 1976, van Aken 2008). On the other hand, the Oosterschelde estuary has a year-round constant salinity around 29.6±4 PSU (Smaal and Nienhuis 1992). *A. rubens* individuals were kept, separated by sampling locality, in two outdoor 4 m^3 tanks containing seawater (salinity = 31 PSU).

Live blue mussels (*Mytilus edulis* L.), collected from subtidal areas in the Wadden Sea (Table 3.1), were used as *A. rubens* prey during the experiment. Mussels were kept in cages hanging from a floating dock at the NIOZ (Royal Netherlands Institute for Sea Research, Texel, The Netherlands) harbour. From these cages batches of mussels were taken to feed *A. rubens.* Mussels had access to natural food in the harbour but no access to food in the outdoor tanks or in the experimental set-up.

Table 6.1. Sampling locality, salinity range and mussel and starfish size details

	Locality & Coordinates	Salinity range at locality (PSU)	Salinity at collection (PSU)	Length (mm)	Weight (g)
Asterias rubens	Oosterschelde (Neeltje Jans) lat: 51.61N lon: 3.72E	28 – 33	31	50.08±13.67	33.49±22.11
	Wadden Sea (Krommebalg) lat: 53.42N lon: 5.71E	20 – 32	30	35.48±7.29	12±6.05
Mytilus edulis	Wadden Sea (Texelstroom) lat: 53.01N lon: 4.82E	20 – 35	30	23.12±6.91	0.28±0.24

3.2.2 Experimental set-up

Experimental set-up consisted of eight recirculation systems (RS), each containing one cubic meter of seawater. Each RS was composed of a buffer tank of 600 l and a shallow (20 cm deep) experimental area (Figure 3.1) placed on top. Biofilters were set at the water input into the experimental areas. After passing through the biofilter, water was distributed into two canals of 25 by 200 cm, each canal contained five circular baskets (25 cm in diameter) made of plastic mesh. Both canals ended in a common compartment that contained two more baskets. After passing this compartment, water was returned to the buffer tank, where it was skimmed and aerated. Water was circulated in the system at a rate of approximately 40 l per minute. RSs were assembled in a temperature-controlled room (13 ºC), a temperature that is within the optimum range of *A. rubens* (Agüera et al. 2012) and *M. edulis* (Saraiva et al. 2012). RSs were covered with a tarpaulin cloth, creating a constantly shaded environment. Shading is known to encourage feeding behaviour and to lower stress levels of *A. rubens* (Agüera et al. 2012). To avoid unwanted effects caused by water quality, pH, nitrites, ammonia and nitrates levels were monitored twice to three times a week. A layer of sand covered the bottom of all experimental areas to approximate substrate conditions in the Wadden Sea.

Each basket in the canals received a clump of mussels, containing approximately 150 mussels, and one starfish (Figure 3.1). In total ten *A. rubens* individuals were set at the canals of each RS (5 from each locality, and 5 in each canal, Figure 3.1). Extra mussels were kept at the space destined for them in each canal (Figure 3.1). Spare specimens of *A. rubens* were placed in the two baskets at the end compartment separated by locality (Figure 3.1).

A. rubens was acclimated to the experimental set-up for three weeks. After acclimation, size of basked individuals was recorded; arm length, measured from the centre of the disk to the arm tip, was measured to the millimetre and weight to the gram. A subset of the starfish was used to determine the correlation between wet weight and ash free dry weights (AFDW) at the start of the experiment; this sample included 24 individuals from each location (total 48). For AFDW determination animals were dried to constant weight at 90 ºC to determine dry weight. Dried animals were then combusted in a furnace for 4 hours at a temperature of 540 ºC to determine ash content. A new batch of mussels was taken from the NIOZ harbour to replace all the mussels kept in the RSs. A day later salinity was decreased, by adding tap water, at a rate of 3 PSU per day until the target treatment salinity was reached. Experimental treatments were: 28, 25, 22, 19, 16, 13, 10 PSU, each one in a different RS. The remaining RS was left as a control at a salinity of 31 (c). A total of 264 starfish divided in 7 different treatments and a control were used. Recording of feeding behaviour and activity started after reaching the target salinity and continued for 22 days (Figure 3.2).

3.2.3 Detachment and mortality

Some treatments caused *A. rubens* to lose grip of the wall of the baskets. This event was recorded. When occurred, regaining of attachment was also noted. Animal mortality was first checked by inactivity (no feeding in 24 hours, no grip) and/or appearance (wasting, body wall perforations, lost arms) and confirmed by touching with a needle the sensorial tube feet of all five arms and closely looking for a reaction. When no reaction was observed the individual was considered dead.

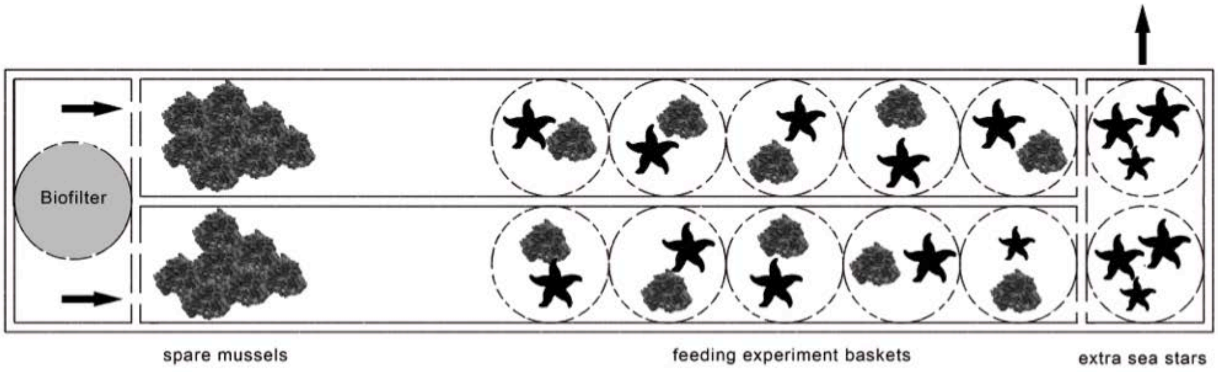
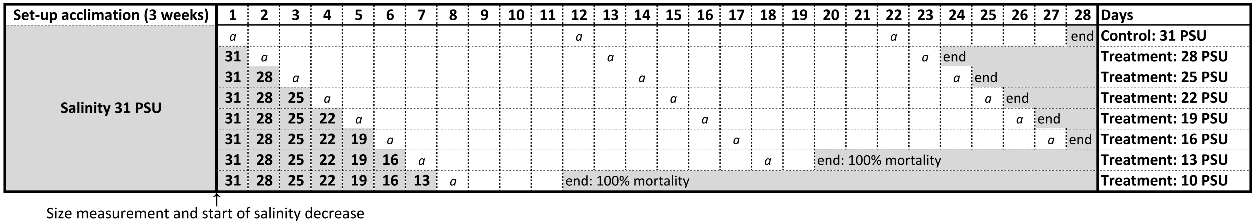


Figure 3.1. Representacion of the experimental area of one recirculation system (RS). Arrows show water direction**.**



Set-up acclimation (3 weeks)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	Days
Salinity 31 PSU	*a*											*a*										*a*						end	**Control: 31 PSU**
	31	*a*											*a*										*a*	end					**Treatment: 28 PSU**
	31	**28**	*a*											*a*										*a*	end				**Treatment: 25 PSU**
	31	**28**	**25**	*a*											*a*										*a*	end			**Treatment: 22 PSU**
	31	**28**	**25**	**22**	*a*											*a*										*a*	end		**Treatment: 19 PSU**
	31	**28**	**25**	**22**	**19**	*a*											*a*										*a*	end	**Treatment: 16 PSU**
	31	**28**	**25**	**22**	**19**	**16**	*a*											*a*		end: 100% mortality									**Treatment: 13 PSU**
	31	**28**	**25**	**22**	**19**	**16**	**13**	*a*				end: 100% mortality																	**Treatment: 10 PSU**

↑ Size measurement and start of salinity decrease

Figure 3.2. Experiment timeline showing timing of measurements and stepwise salinity decrease for earch RS independently. *a* represents activity measurements. The control was continued until all treatments were terminated

3.2.4 Activity coefficient

Asterias rubens activity coefficient, as described in (Shirley and Stickle 1982) was measured in individuals from all treatments at day 1, 12 and 22. Activity was calculated by dividing 1000 by the time in seconds required for an individual starfish to move its disk to a vertical position after being placed on its aboral side on a horizontal surface under water. This index was determined using specimens kept in the baskets containing extra specimens to avoid handling of animals under observation for feeding behaviour. A total of 8 individuals were used in each treatment (4 from each site) every time activity was measured. Activity was always measured in different individuals. Size (weight in grams and arm length in mm) of individuals used to determine activity was recorded.

3.2.5 Feeding behaviour

Experimental individuals were monitored on a daily basis. Feeding rate of each specimen placed in an individual basket was recorded every day. Feeding frequency was recorded as percentage of animals that fed between feeding rate recordings (every 24 hours). Eaten mussels, collected from the baskets using long forceps, were counted and their shell length measured to the nearest millimetre. Eaten mussels were replaced by live mussels of the same size from the extra mussels stored in the same treatment RS trying to keep the length distribution of available prey as constant as possible during the experiment. Handling of the individuals placed in baskets was reduced to a minimum. It was not possible to determine the mass of eaten mussels directly as only the shells remained. To determine it indirectly from shell length a reference sample of mussels was taken each week from each RS. These mussels were measured to nearest 0.1 millimetre. Soft tissue was removed from the shell and weighed to the nearest 0.001 g. Ash free dry weight (AFDW) was then obtained following the protocol by Kamermans et al. (2009).

3.2.6 Growth

At the end of the experiment (day 22) all surviving starfish placed in the individual baskets were measured for arm length, wet weight and AFDW. Growth was then obtained as the difference in AFDW measured at the end and the approximated (from the measured reference group at the start of the experiment) AFDW before the salinity decrease started. Growth in arm length and wet weight was determined in a similar manner. For treatments with salinity 25 PSU and below growth included the time needed to decrease salinity to the desired treatment, average daily growth was used in the analyses.

3.2.7 Data analyses

Multiregression linear models were fitted to describe the effect of salinity, time (time in days since target treatment salinity was reached), starfish size and locality in the measured variables: activity, feeding rate, feeding frequency, consumed mussel size and growth. In all cases Akaike Information Criterion and Log-likelihood was used to select the best model. Data on feeding rate and prey size resulted from repeated measurements taken from the same individuals over time, in those cases individual starfish identification was used in the linear models as random factor. This approach was more convenient than two-way (repeated measurements) ANOVA, due to the characteristics of the data and the flexibility offered by linear mixed effect models.

Activity was analysed using a linear regression model. Initially considering it a function of salinity, individual size, time, locality and their interactions. Activity data variance increased with salinity, therefore a weighed linear model was used (function lme, with weights as: varPower(), R v.2.13, package: nlme).

Linear multiregresion models were fitted to describe log-transformed mussel AFDW and soft tissue wet weight as a function of mussel shell length, salinity and time. The resulting models explained over 95% of the variance of AFDW and soft tissue wet weight of sampled mussels as a function of mussel log transformed length. Those models were used to transform eaten mussel lengths to consumed mussel soft tissue wet weight and AFDW. Feeding rate was standardized by individual starfish wet weight and measured as gram mussel dry weight per gram starfish wet weight per day. This standardization eliminated the effect of size in feeding rate (Agüera et al. 2012). However starfish in different treatments exhibited different growth, and individual size affected the observed feeding rate during the 22 days the experiment lasted. It was necessary to eliminate the effect of differential growth between treatments on individual daily feeding rate. Weight of each individual at each day was updated considering the observed growth in wet weight of each *A. rubens* during the experiment. Daily feeding rate was then standardized by that approximated weight.

Effects of salinity and time on feeding rate as gram of mussel soft tissue wet weight per day and gram of *A. rubens* wet weight, were assessed using a linear mixed effect statistical model. Because variance in feeding rate increased with salinity, we used a weighted linear regression, such that variance increased as a power function of salinity (function lme, with weights as: varPower(), R v.2.13, package: nlme). As feeding rate data resulted from repeated observations of the same individuals during the experiment, individual identification was used in the linear model as random effect. Additionally to describe when animals started to feed and the change in feeding rate over time, linear and log-log models were fitted to feeding rates as function of time for each treatment.

Feeding frequency was described using a linear model with binomial distribution (function glm, family: binomial, R v.2.13).

The effect of salinity treatment and exposure time on eaten mussel shell lengths were also described using a linear mixed effect statistical model (function lme, R v.2.13, package: nlme). As they resulted from repeated measuring, individuals were added as a random effect to the model.

Growth was recorded as change in AFDW over the experimental period. Effect of salinity and feeding rate (as grams of mussel AFDW consumed) was analysed by means of linear regression of log-transformed feeding rate against log-transformed time. Best fitting model was chosen comparing resulted R-square values. As both salinity and feeding rate exhibited a strong colinearity, no analysis involving both variables at the same time was considered.

Final model details and validation plots are presented in Appendix 3.1.

3.3 Results

Water quality was kept constant and under good conditions for the whole experiment with pH ranging between 7.9 and 8.0; ammonia and nitrite levels were kept at 0 mg l^{-1}. In the results, multiregressions model slopes are given to indicate the size of the change resulted from each of the variables considered. Further data on slopes, significance and model fit (AIC, residual plots) can be found in Appendix 3.1.

3.3.1 Mortality and detachment

Decreasing salinity from 31 PSU to 10 PSU and 13 PSU resulted in 100% mortality within 4 and 13 days respectively, with no difference between locations. Two animals died in treatments at 19 and 16 PSU, while only one dead animal was counted at salinity 25 PSU. No mortality was recorded in any other treatment. Additionally no natural mortality of mussels was recorded in any treatment, including those where *A. rubens* mortality reached 100%.

At 16 PSU, 50% of the individuals detached from the basket walls and were observed upside down on the bottom at day 1. All of them regained their position during the first week of the experiment. In treatments 13 PSU and 10 PSU 100% of individuals were detached at day 1 and never regained attachment. No detachment was observed in any of the other treatments.

3.3.2 Activity

Activity decreased with salinity (Figure 3.3A) (multiregression model, slope = 0.655, *p*-value < 0.001). Activity coefficient was lower and salinity effect stronger in larger animals (slope = $-2.466\ 10^{-3}$, p-value = 0.011). An increase of activity was observed over time in all the treatments with survivors, except for the control (31 PSU). This was also described by the multiregression model as a significant effect of time (slope = 0.087, *p*-value < 0.001) in the output (Figure 3.3B). No difference between localities was observed.

3.3.3 Feeding behaviour

Decreasing salinity negatively affected feeding activity. Osmotic stress resulted in a reduction of individuals feeding rate and also on feeding frequency.

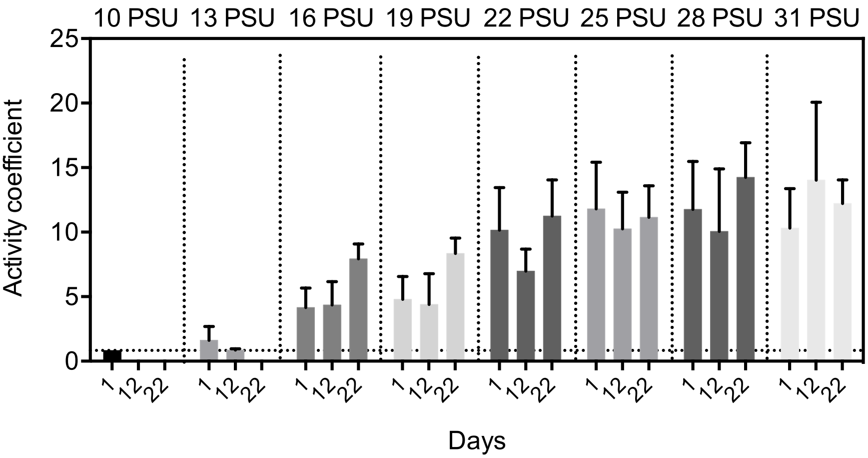


Figure 3.3. Activity coefficient by salinity treatment and exposure time. Error bars are standard deviation, n = 8 in all bars.

The fitted multiregression model described the role of salinity and time in feeding rate. In this model salinity has a significant effect (Figure 3.4A) (slope = 1.768 10^{-3}, *p*-value < 0.001), and also time (Figure 3.4B) (slope = -8.671 10^{-4}, *p*-value < 0.001) and the interaction between salinity and time was significant (slope = 5.564 10^{-5}, p-value < 0.001). The overall effect of time resulted in a recovery of feeding rate after first exposure. This recovery was steeper in the treatments where animals stopped feeding at the start of the exposure to the decreased salinity (Figure 3.4B). No difference in feeding rate was observed between starfish from Wadden Sea and Oosterschelde.

Individuals in treatments 16 PSU and 19 PSU stopped feeding for more than 24 hours at the start of the experiment. Individuals at 13 PSU and 10 PSU never fed before dying. Salinity also reduced the feeding frequency in the other treatments, with less animals being observed feeding during a day (Figure 3.4C and 3.4D). As the experiment progressed, animals in treatment 19 restarted feeding, followed later by animals in treatment 16 (Figure 3.4B).

Although all *A. rubens* individuals used in this study were able to feed on the whole range of mussel lengths offered, a significant effect of salinity on the consumed mussel length was found (Figure 3.4E) (slope = 0.014, *p*-value < 0.001). An increase in the length of consumed mussels over time was observed in treatments 25, 22 and

19 as the experiment lasted (Figure 3.4F). Consumed mussel size depended also on *A. rubens* size (arm length). A significant effect of locality (slope = -0.2811, *p*-value < 0.01) was found on consumed mussel size. Starfish of the same size consumed larger mussels when coming from the Wadden Sea than those coming from the Oosterschelde. The interaction between locality and *A. rubens* size was also significant (slope = 0.0045, *p*-value < 0.01).

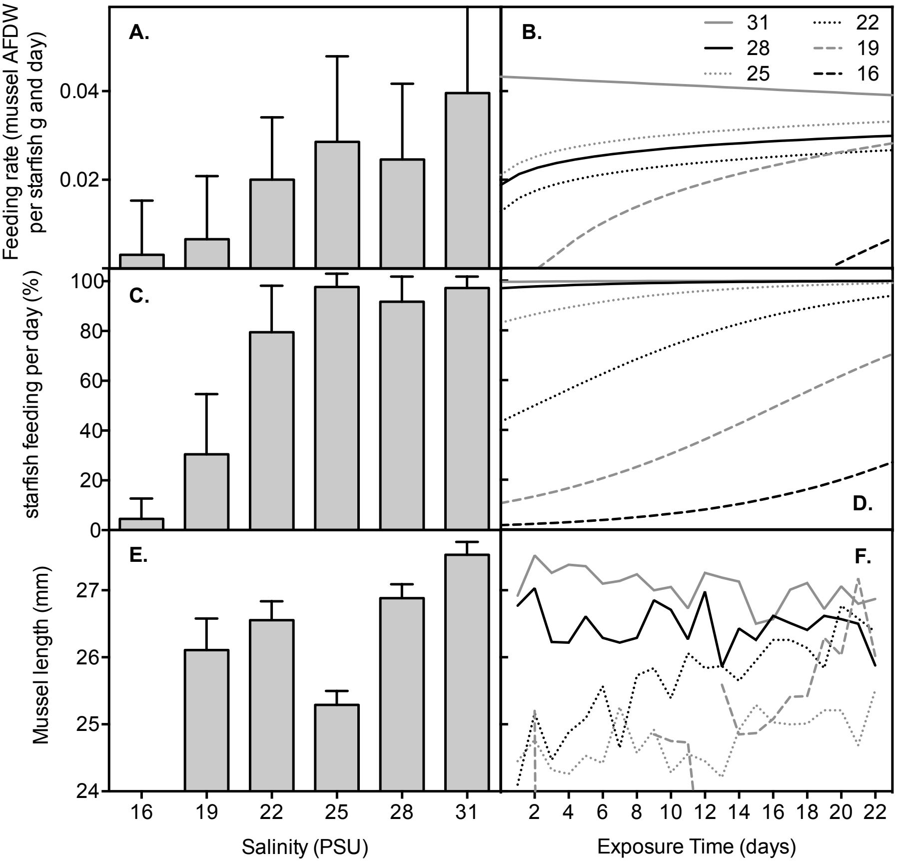


Figure 3.4. Starfish feeding behaviour and acclimation in the different salinity treatments. **A.** daily feeding rate by salinity. **B.** changes in daily feeding rate with exposure time, lines are linear and log-log regressions output (see appendix I for details). **C**. feeding frequency as percentage of starfish feeding per day and salinity. **D.** feeding frequency acclimation with exposure time, lines are output from glm with binomial distribution (see appendix 3.1). **E.** length of consumed mussels at each salinity treatment. **F.** mean length of consumed mussel per day (lines are connects mean of consecutives days). All bar plots are mean and standard deviation (error bar). Legend in plot B. applies to all line plots.

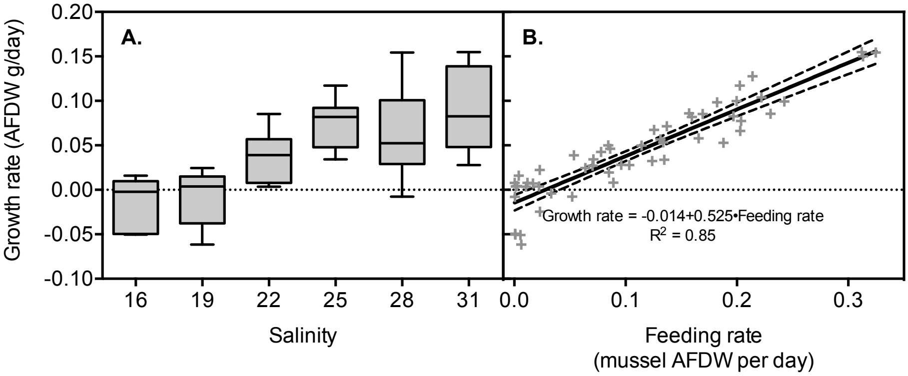


Figure 3.5. Starfish growth. **A.** growth in each salinity treatment, n = 8 for 16 and 19 PSU, n = 9 for 25 PSU, n = 10 for the rest. Box-plots with median and Tukey error bars. **B.** growth as a function of feeding rate. Line is regression output. Slashed lines represent 95% confident interval, n = 55.

3.3.4 Growth

Recording of growth was only possible in treatments with survivors at the end of the experiment. Growth in AFDW was found to be less variable than arm length or wet weight. Growth rate (grams AFDW per day) was slower at lower salinities, with negative growth being observed in 19 and 16 PSU treatments (figure 3.4). Besides the clear salinity effect, growth was better described as a function of feeding rate in AFDW of mussel consumed per day (figure 3.4) (*F*-value=299.4, *p*-value < 0.001, R-square = 0.85).

3.4 Discussion

Water quality was maintained at good conditions in all RSs. Feeding rate observed in the control was higher than those obtained in previous studies using similar conditions in open circulation systems (Agüera et al. 2012). In treatments at 28 PSU and 25 PSU, feeding rate was close to the maximum values reported by Agüera et al. (2012). No possible effects of deteriorating water quality were observed in any RS. The decrease of feeding rate observed in the control (31 PSU) (Figure 3B) was probably due to the decrease in feeding rate by starfish biomass gram with the increase of individual starfish size (Agüera et al. 2012).

The osmotic stress gradient tested in this study showed that salinity decreases, such as those that may take place when sudden freshwater input events occur (Zimmerman 1976, Kashenko 2003), have an important role determining survival and performance of *A. rubens*. An important reduction of performance was observed. The magnitude of this reduction increased with osmotic stress, resulting in 100% mortality at the higher levels. Acclimation was observed in all treatments where *A. rubens* individuals survived, with feeding rate and activity improving over time. The effects of salinity decreases on performance and distribution of *A. rubens* have an important role in determining the impact of this predator in cultured and natural mussel beds located in areas where salinity exhibits this type of variability, such as the Wadden Sea. Besides the previously studied link between *A. rubens* survival and response to local salinity regimes (Binyon 1961, Saranchova 2001), the present study did not find any differences in survival between individuals sourced from two locations with different salinity regimes.

3.4.1 Survival and salinity decrease timescale

Previous experiments with immediate salinity changes observed survival of *A. rubens* to changes of 8-10 PSU (Binyon 1961; Saranchova 2001) when acclimated to salinities between 31 PSU (Binyon 1961; Saranchova 2001) and 24 PSU (Saranchova 2001). In this study we observed that, despite its poor capacity for osmoregulation (Binyon 1961, Shumway 1977), *A. rubens* was able to endure and survive a salinity decrease down to 16 PSU from an acclimation salinity of 31 PSU, that is a change of 15 PSU. Importantly, our experimental salinity was decreased in a stepwise manner. This slower salinity decrease rate may increase the tolerance range of *A. rubens.* Thus *A. rubens* survival to a salinity decrease appears to depend on both how large the change is and how fast it occurs. Differences in minimum sustainable salinity between experimental *A. rubens* groups has previously been attributed to adaptations to local salinity regimes (Saranchova 2001). However, in the present study, no difference in mortality was observed between *A. rubens* sourced from two different locations, one with all year round constant salinity and another with a highly variable (seasonally and tidally) salinity. This result could mean that the acclimation period spent at 31 PSU by experimental individuals in this study was enough to negate any adaptation to local salinity conditions. Alternatively, local differences encountered in previous studies (Binyon 1961, Saranchova and Lukanin 1989, Saranchova 2001) may be due to genetic differences among populations, all *A. rubens* individuals used in this study are thought to come from a single genetic population (Agüera et al. 2012).

3.4.2 Limits to performance and acclimation

Besides the capacity of *A. rubens* to endure stepwise changes up to 15 PSU in salinity, the effect of osmotic stress on its performance was already noticeable with a reduction from 31 to 28 PSU. The decrease of salinity resulted in an immediate reduction of activity and feeding rate. In treatments where salinity was changed at least 12 PSU (treatments with salinity 19 PSU and lower) all individuals stopped feeding for some time. The effect of salinity on individual growth of *A. rubens* has been observed previously (Guillou et al. 2012). In other starfish species, a reduction of feeding rate and activity have been described as an effect of osmotic stress (Shirley and Stickle 1982, Russell 2013). In the case of *A. rubens,* osmotic stress produces a reduction in the attachment strength (Kashenko 2006) and an increase in body water content (Binyon 1961). Loss of attachment strength results in a reduced activity coefficient and capacity to deliberately move and feed. Increases of internal pressure, due to osmotic stress, may result in cellular and tissue damage (Binyon 1976), inhibiting movement and requiring time to recover. The resulting observed reduction of feeding and activity may be a consequence of physical limitations caused by increased body water and loosening of attachment strength.

As seen in the present study, attachment strength is regained and internal water volumes are normalized over time (Binyon 1961). Signs of acclimation were observed in all the treatments where the animals survived, with feeding being resumed and feeding frequency increasing over time. However, larger decreases in salinity resulted in disproportionate increases in the acclimation time of *A. rubens*, delaying the resumption of feeding and slowing the increase in feeding frequency. By the end of the experiment feeding rate and activity increased in all treatments with a salinity of 22 PSU or higher, tending towards that of the control group (31PSU). This suggests that, given enough time to acclimate, animals in lower salinities can perform as well as those at 31 PSU. Acclimation to changes in environmental salinity has been observed in other echinoderms, however is often a slow process that requires longer-term experiments (Russell 2013). It remains unknown if *A. rubens* individuals completely acclimated to 22 PSU would be able to survive an additional decrease in salinity of the same magnitude, or whether acclimation time would be similar, slower or performance would be permanently reduced. It is noteworthy that populations of *A. rubens* exist down to 8 PSU with individuals even surviving at 4 PSU (Kowalski 1955). This suggests that where 100% mortality occurred in the experiment presented here, acclimation may still have occurred if salinity changes had been slower. Importantly, survival and posterior acclimation will depend on the timescale of the salinity decrease.

The physical or physiological limitation imposed on feeding by osmotic stress has an impact on growth. For example, in the treatment at 22 PSU, the decrease in salinity resulted in a 50% reduction in feeding rate and growth during the three experimental weeks compared to the control at 31 PSU. It is further conceivable that reduced growth may be a consequence of reduced digestion efficiency due to metabolic limitations imposed by osmotic stress (Shirley and Stickle 1982, Forcucci and Lawrence 1986). The present work did not study digestion efficiency, however, results imply that reduced growth is a direct consequence of limitations to feeding.

Our results further show that osmotic stress has a direct effect on the behaviour of *A. rubens* and the interaction with its prey. After salinity was decreased, experimental animals selected smaller mussels, then, progressively selected bigger prey as they acclimated to the new environment. This effect of osmotic stress in prey selection has been observed before in other marine invertebrates such as crabs (Aronhime 2010) where crabs shortened handling time of prey when exposed to osmotic stress. For *A. rubens,* smaller mussels requires less time to handle and less strength compared to larger mussels (O'Neill et al. 1983, Hummel et al. 2011). Selection differed between locations, with *A. rubens* from the Wadden Sea feeding on larger mussels than *A. rubens* of equivalent size from the Oosterschelde. This study was unable to disentangle the reasons behind such differentiation. There is a possibility that it may be a consequence of adaptation to local salinity or a result from previous feeding experience at their origin site (O'Neill et al. 1983), as no other difference between locations has been found in this study.

3.4.3 Ecological implications for mussel populations

The effects of osmotic stress limiting the performance and causing mortality of *A. rubens* have a direct consequence on its role as a keystone predator of mussels. Subtidal mussel beds are often found in areas were salinity variability is high both seasonally and tidally. Mussels are known to be more resilient to salinity changes than *A. rubens* (Saranchova and Lukanin 1989). This was also seen in the present study, where no mortality of mussels was recorded in any treatment as a result of salinity. Therefore mortality in areas were salinity changes are large and fast enough to cause mortality of *A. rubens,* but not of blue mussels, a reduction of predation pressure will improve survival of the latter. Thus, mussels may temporally or permanently escape *A. rubens* predation.

Asterias rubens can survive moderate levels of osmotic stress and acclimate to new conditions. However, the acclimation process is slow resulting in significant differences in the amount of mussels consumed and *A. rubens* individual growth across salinity treatments. Altogether, decreased feeding, slower or negative individual growth and the consumption of smaller prey during the acclimation process may allow mussels to escape predation by attaining physical size refuge. Although mussel growth is also affected by salinity (Almada-Villela 1984, Westerbom et al. 2002), mussels are better adapted to withstand osmotic stress than *A. rubens* (Saranchova and Lukanin 1989) allowing them to outgrow *A. rubens* predation size range. The final result of this temporal refuge and its consequences will depend on timing and on the magnitude of the change in salinity. The temporal reduction of predation pressure will be more important shortly after settlement when mussels exhibit larger growth rates (Saraiva et al. 2012).

As a mobile predator *A. rubens* may also scape low salinity areas by moving away. This behaviour has been observed in other starfish species (Barker and Russell 2008, Lamare et al. 2009). Moving back and forward following salinity changes. This behaviour results in a decrease of the predation level in the areas affected by low salinity, as the predator is temporally excluded. However *A. rubens* cannot move fast, and this mechanism may be only useful when bottom topography and water mixing process permit to scape a low salinity layer by moving relatively short distances (Barker and Russell 2008). In the case that is not possible or changes are too sudden and large, starfish will resort to low activity levels (Barker and Russell 2008) and acclimation.

We concluded that osmotic stress due to changes in salinity plays an important role in the distribution and performance of *A. rubens* and is an important factor controlling the impact of this predator in mussel populations. Although *A. rubens* can acclimate, hampered predation performance impacts directly on its role as a shellfish predator and therefore has an impact on the survival of its prey population. At high stress levels, i.e. areas where salinity changes are large and/or fast, *A. rubens* may be excluded as the prey attains spatial refuge. At lower stress levels, i.e. areas were salinity changes are smaller and/or slow, *A. rubens* may persist and acclimate but its performance would be diminished. Timescale is important for both lethal and sublethal effects, as they depend on how large and how fast the salinity change is.

References

Agüera, A., M. Trommelen, F. Burrows, J. M. Jansen, T. Schellekens, and A. Smaal. 2012. Winter feeding activity of the common starfish (*Asterias rubens* L.): The role of temperature and shading. Journal of Sea Research 72:106–112.

Almada-Villela, P. C. 1984. The effects of reduced salinity on the shell growth of small *Mytilus edulis*. Journal Of The Marine Biological Association Of The United Kingdom 64:171–182.

Aronhime, B. R. 2010, April 21. Predator-Prey interaction in estuarine bivalves: size selection, effects of salinity , and indirect interactions. Ph.D Thesis. University of North Alabama.

Barbeau, M. A., and R. E. Scheibling. 1994. Behavioral mechanisms of prey size selection by sea stars (*Asterias vulgaris* Verrill) and crabs (*Cancer irroratus* Say) preying on juvenile sea scallops (*Placopecten magellanicus* (Gmelin)). The Journal of Experimental Marine Biology and Ecology 180:103–136.

Barker, M. A. and R. E. Scheibling, 1994. The distribution and behaviour of *Patiriella mortenseni* and *P. regularis* in the extreme hyposaline conditions of the Southern New Zealand Fjords. Journal of Experimental Marine Biology and Ecology 355: 76-84.

Bertness, M. D., and E. Grosholz. 1985. Population dynamics of the ribbed mussel, Geukensia demissa: The costs and benefits of an aggregated distribution. Oecologia 67:192–204.

Binyon, J. 1961. Salinity Tolerance and Permeability to Water of the Starfish *Asterias rubens* L. Journal Of The Marine Biological Association Of The United Kingdom 41:161–174.

Binyon, J. 1962. Ionic Regulation and Mode of adjustment to Reduced Salinity of the Starfish *Asterias rubens* L. Journal Of The Marine Biological Association Of The United Kingdom 42:49–64.

Binyon, J. 1976. The effects of reduced salinity upon the starfish *Asterias rubens* L. together with a special consideration of the integument and its permeability to water. Thalassia Jugoslavica 12:11–20.

Brun, E. 1968. Extreme population density of the starfish *Asterias rubens* on a bed of Iceland scallops. Astarte 32:1–3.

Bruno, J. F. 2001. Habitat modification and facilitation in benthic marine communities. Pages 201–218 *in* S. D. Gaines, M. D. Bertness, and M. E. Hay, editors. Marine Community Ecology. First. Sinauer Associates, Inc, Sunderland.

Burnaford, J. L. 2004. Habitat modification and refuge from sublethal stress drive a marine plant-herbivore association. Ecology 85:2837–2849.

Forcucci, D., and J. M. Lawrence. 1986. Effect of low salinity on the activity, feeding, growth and absorption efficiengy of *Luidia clathrata* (Equinodermanta: Asteroidea). Marine Biology 92:315–321.

Guillou, M., G. Joly-Turquin, S. Leyzour, P. Pernet, and P. Dubois. 2012. Factors controlling juvenile growth and population structure of the starfish Asterias rubens in intertidal habitats: field and experimental approaches. Journal Of The Marine Biological Association Of The United Kingdom 92:367–378.

Hancock, D. A. 1955. The Feeding Behaviour of Starfish on Essex Oyster beds. Journal Of The Marine Biological Association Of The United Kingdom 34:313–331.

Hart, D. R. 2006. Effects of sea stars and crabs on sea scallop *Placopecten magellanicus* recruitment in the Mid-Atlantic Bight (USA). Marine Ecology Progress Series 306:209–221.

Hummel, C., P. Honkoop, and J. van der Meer. 2011. Small is profitable: No support for the optimal foraging theory in sea stars *Asterias rubens* foraging on the blue edible mussel *Mytilus edulis*. Estuarine, Coastal and Shelf Science 94:89–92.

Kamermans, P., M. Blankendaal, and J. Perdon. 2009. Predation of shore crabs (*Carcinus maenas* (L.)) and starfish (*Asterias rubens* L.) on blue mussel (*Mytilus edulis* L.) seed from wild sources and spat collectors. Aquaculture 290:256–262.

Kashenko, S. D. 2003. The Reaction of the Starfish *Asterias amurensis*; and Patiria pectinifera (Asteroidea) from Vostok Bay (Sea of Japan) to a Salinity Decrease. Russian Journal of Marine Biology 29:110–114–114.

Kashenko, S. D. 2006. The combined effect of temperature and salinity on development of the sea star Asterina pectinifera. Russian Journal of Marine Biology 32:37–44.

Kowalski, R. 1955. Untersuchungen zur Biologie des Seesternes Asterias rubens L. in Brackwasser. Kieler Meeresforschungen XI.

Lamare, M. D., T. Channon, C. Cornelisen and C. Murray, 2009. Archival electronic tagging of a predatory sea star – Testing a new technique to study movement at the individual level. The Journal of Experimental Marine Biology and Ecology 373:1–10.

Magnesen, T., and K. J. Redmond. 2011. Potential predation rates by the sea stars Asterias rubens and Marthasterias glacialis, on juvenile scallops, Pecten maximus, ready for sea ranching. Aquaculture International 20:189–199.

Menge, B. A. 1978. Predation intensity in a rocky intertidal community. Effect of an algal canopy, wave action and desiccation on predator feeding rates. Oecologia 34:17–35.

Menge, B. A. 1982. Effects of feeding on the environment: Asteroidea. Pages 521–551 *in* M. Jangoux, J. M. Lawrence, and J. M. Lawrence, editors. Echinoderm Nutrition. A.A. Balkema, Rotterdam.

Menge, B. A., and J. P. Sutherland. 1976. Species diversity gradients: Synthesis of the roles of predation, competition and temporal heterogeneity. The American Naturalist 110:351–369.

Menge, B. A., and J. P. Sutherland. 1987. Community Regulation: Variation in Disturbance, Competition, and Predation in Relation to Environmental Stress and Recruitment. The American Naturalist 130:730–757.

Nauen, C. E. 1978. The growth of the sea star, *Asterias rubens*, and its role as benthic predator in Kiel Bay. Kieler Meeresforschungen 4:68–81.

O'Neill, S., A. Sutterlin, and D. Aggett. 1983. The effects of size-selective feeding by starfish (*Asterias vulgaris*) on the production of mussels (*Mytilus edulis*) cultured on nets. Aquaculture 35:211–220.

Robles, C. D., R. A. Desharnais, C. Garza, M. J. Donahue, and C. A. Martinez. 2009. Complex equilibria in the maintenance of boundaries: experiments with mussel beds. Ecology 90:985–995.

Russell, M. P., 2013. Echinoderm responses to variations in salinity. Advances in Marine Biology 66:171-213.

Saraiva, S., J. Van der Meer, S. A. L. M. Kooijman, R. Witbaard, C. J. M. Philippart, D. Hipple, and R. Parker. 2012. Validation of a Dynamic Energy Budget (DEB) model for the blue mussel Mytilus edulis. Marine Ecology Progress Series 463:141–158.

Saranchova, O. L. 2001. Research into tolerance for the environment salinity in sea starfish *Asterias rubens* L. from populations of the White Sea and Barentz Sea. The Journal of Experimental Marine Biology and Ecology 264:15–28.

Saranchova, O. L., and L. P. Flyachinskaya. 2001. The Influence of Salinity on Early Ontogeny of the Mussel *Mytilus edulis* and the Starfish *Asterias rubens* from the White Sea. Russian Journal of Marine Biology 27:87–93.

Saranchova, O. L., and V. V. Lukanin. 1989. Adaptive reponses in the starfish *Asterias rubens* and the blue mussel *Mytilus edulis* L. from the White Sea to salinity changes. Biologiya Morya-Marine Biology:29–35.

Shirley, T. C., and W. B. Stickle. 1982. Responses of *Leptasterias hexactis* (Echinodermata : Asteroidea) to Low Salinity. I. Survival, Activity, Feeding, Growth and Absorption Efficiency. Marine Biology 69:147–154.

Shumway, S. E. 1977. The effects of fluctuating salinities on four species of asteroid echinoderms. Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 58A:177–179.

Smaal, A. C., and P. H. Nienhuis. 1992. The eastern Scheldt (The Netherlands), from an estuary to a tidal bay: A review of responses at the ecosystem level. Netherlands Journal of Sea Research 30:161–173.

Stickle, W. B., M. N. Moore, and B. L. Bayne. 1985. Effects of temperature, salinity and aerial exposure on predation and lysosomal stability of the dogwhelk Thais (Nucella) lapillus (L.). The Journal of Experimental Marine Biology and Ecology 93:235–258.

Strom, S. L., E. L. Harvey, K. A. Fredrickson, and S. Menden-Deuer. 2012. Broad Salinity Tolerance as a Refuge from Predation in the Harmful Raphidophyte Alga Heterosigma akashiwo(Raphidophyceae). Journal of Phycology 49:20–31.

van Aken, H. M. 2008. Variability of the salinity in the western Wadden Sea on tidal to centennial time scales. Journal of Sea Research 59:121–132.

Westerbom, M., M. Kilpi, and O. Mustonen. 2002. Blue mussels, Mytilus edulis , at the edge of the range: population structure, growth and biomass along a salinity gradient in the north-eastern Baltic Sea. Marine Biology 140:991–999.

Wing, S. R., and J. J. Leichter. 2011. Variation in environmental conditions in a subtidal prey refuge: effects of salinity stress, food availability and predation on mussels in a fjord system. Marine Ecology Progress Series 422:201–210.

Witman, J. D. and K. R. Granger. 1998. Links between rain, salinity, and predation in a rocky subtidal community. Ecology 79:2429-2447.

Witman, J. D., S. J. Genovese, J. F. Bruno, J. W. McLaughlin, and B. I. Pavlin. 2003. Massive Prey Recruitment and the Control of Rocky Subtidal Communities on Large Spatial Scales. Ecological Monographs 73:441–462.

Wolff, W. J. 1968. The Echinodermata of the Estuarine region of the Rivers Rhine, Meuse, and Scheldt, with a list of species occurring in the Coastal Waters of the Netherlands. Netherlands Journal of Sea Research 4:59–85.

Zimmerman, J. T. F. 1976. Mixing and flushing of tidal embayments in the western Dutch Wadden Sea.

Part I: Distribution of Salinity and calculation of mixing time scales. Netherlands Journal of Sea Research 10:149–191.

Appendix 3.1 Models outputs and validation plots

Appendix 3.1.1. General Linear Model for *Asterias rubens* activity

Variables:

WWeight	Starfish wet weight
Sal	Salinity Treatment
Day	Experimental day

General Linear Model: Activity = $a + b_1 \cdot \text{Sal} + b_2 \cdot \text{Day} + b_3 \cdot \text{WWeight}$

AIC	BIC	LogLik
777.82	796.1	-382.91

Deviance results

Min	1Q	Median	3Q	Max
-9.8822	-1.6501	0.0718	1.5315	7.7237

Coefficients:

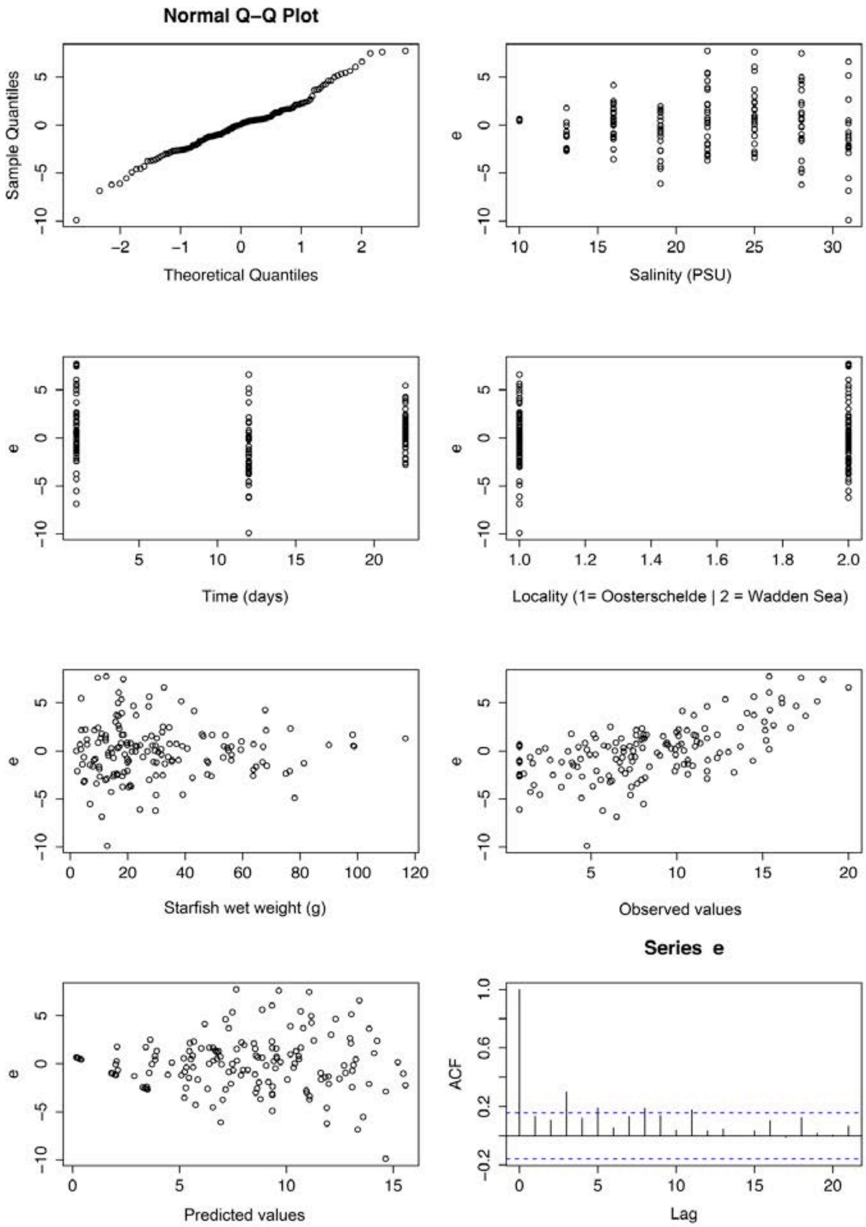
	Estimate	Std. Error	t value	p-value
Intercept	(a) -4.43982	0.88388	-5.023	0
Sal	(b_1) 0.56870	0.03829	14.852	0
Day	(b_2) 0.12284	0.02855	4.303	0.0003
WWeight	($b3$) -0.03674	0.01055	-3.482	0.0006

(Dispersion parameter for Gaussian family taken to be 8.1972

	Value	Degrees of freedom
Null deviance	3495.2	155
Residual deviance	1237.8	151

Number of Fisher Scoring iterations: 2

Residual plots: (e = Pearson’s residuals)



Appendix 3.1.2. Linear mixed effects model for *Asterias rubens* feeding rate

Variables:

FRw	Feeding rate as grams mussel flesh per starfish gram
Sal	Salinity Treatment
Day	Experimental day
ID	Individual starfish identification number

Linear mixed-effects model fit by REML

AIC	BIC	LogLik
-8024.28	-7972.95	4022.14

Random Effects: Formula: ~1 | ID

	Intercept	Residual
StdDev	0.0084	0.1736

Correlation Structure: ARMA(0,3)

Parameter estimates

Theta 1	Theta 2	Theta 3
0.0763	0.1292	0.08623

Variance function: Structure: Power of variance covariate. Power: 0.6156

Fixed Effects: FRw ~ Sal • Day

Coefficients:

	Estimate	Std. Error	DF	t-value	p-value
Intercept	-0.0277	0.0057	1197	-4.8685	0
Sal	0.0017	0.0002	56	7.0253	0
Day	-0.0008	0.0001	1197	-5.8449	0
Sal • Day	0.00005	0.000001	1197	6.1465	0

Correlation:

	Intercept	Sal	Day
Sal	-0.972		
Day	-0.263	0.361	
Sal • Day	0.263	-0.363	-0.996

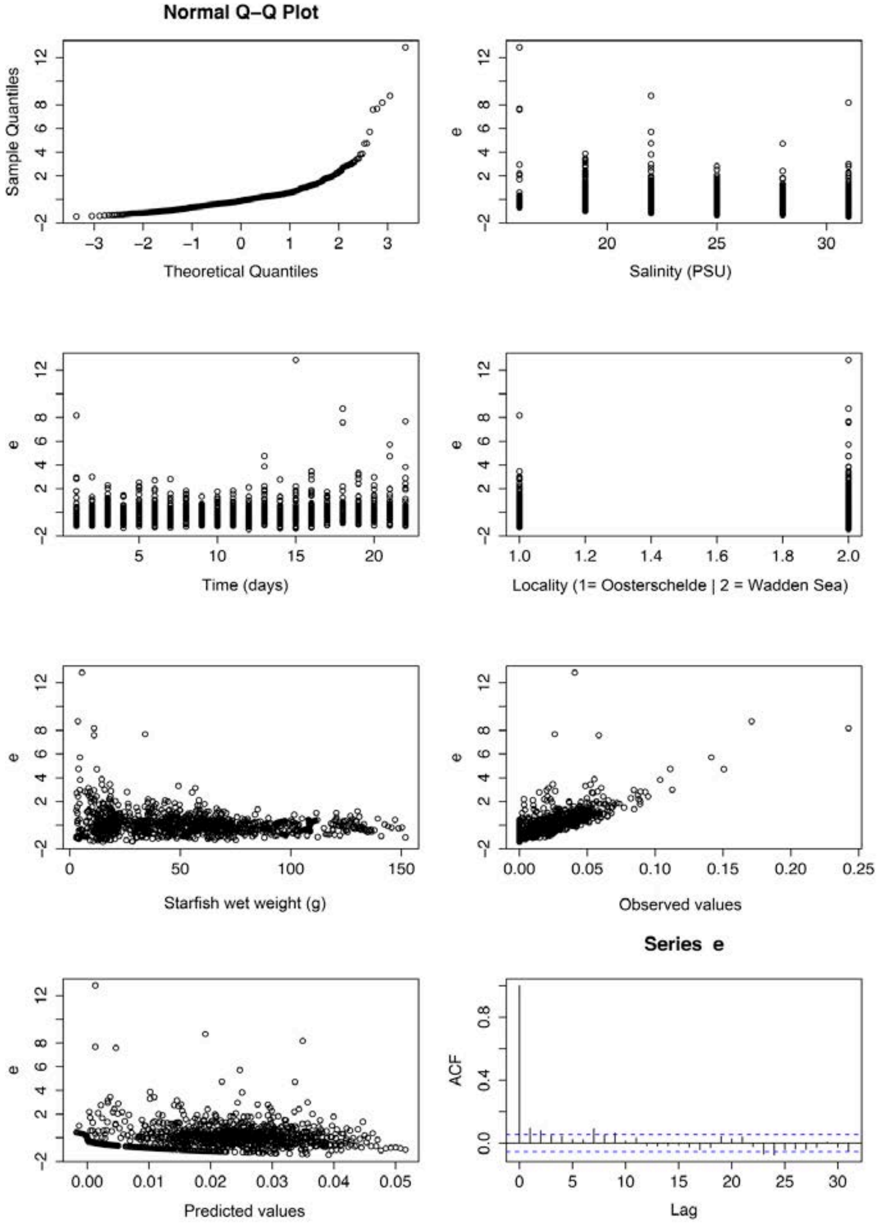
Standardized Within-Group Residuals:

Min	1Q	Median	3Q	Max
-1.7781	-0.5126	-0.0952	0.3971	11.8791

Number of observations: 1257

Number of groups: 58

Residual plots: (e = Pearson's residuals)



Appendix 3.1.3. General linear model (binomial family) for *Asterias rubens* feeding frequency

Variables:

FF	Feeding activity (1: feeding, 0: not feeding)
Sal	Salinity Treatment
Day	Experimental day
ID	Individual starfish identification number

Generalized Linear mixed-effects model fit by maximum likelihood (Laplace Approx.)

Family: Binomial (logit)

AIC	BIC	LogLik
66931	690	-330.6

Random Effects: Formula: ~1 | ID

	Intercept	Residual
StdDev	3.875	1.968

Fixed Effects:

Coefficients:

	Estimate	Std. Error	DF	z	p-value
Intercept	-15.6598	1.69875	1301	-9.218	0
Sal	0.6623	0.0718	58	9.221	0
Day	0.1793	0.0206	58	8.699	0

Correlation:

	Intercept	Sal
Sal	-0.977	
Day	-0.383	0.283

Standardized Within-Group Residuals:

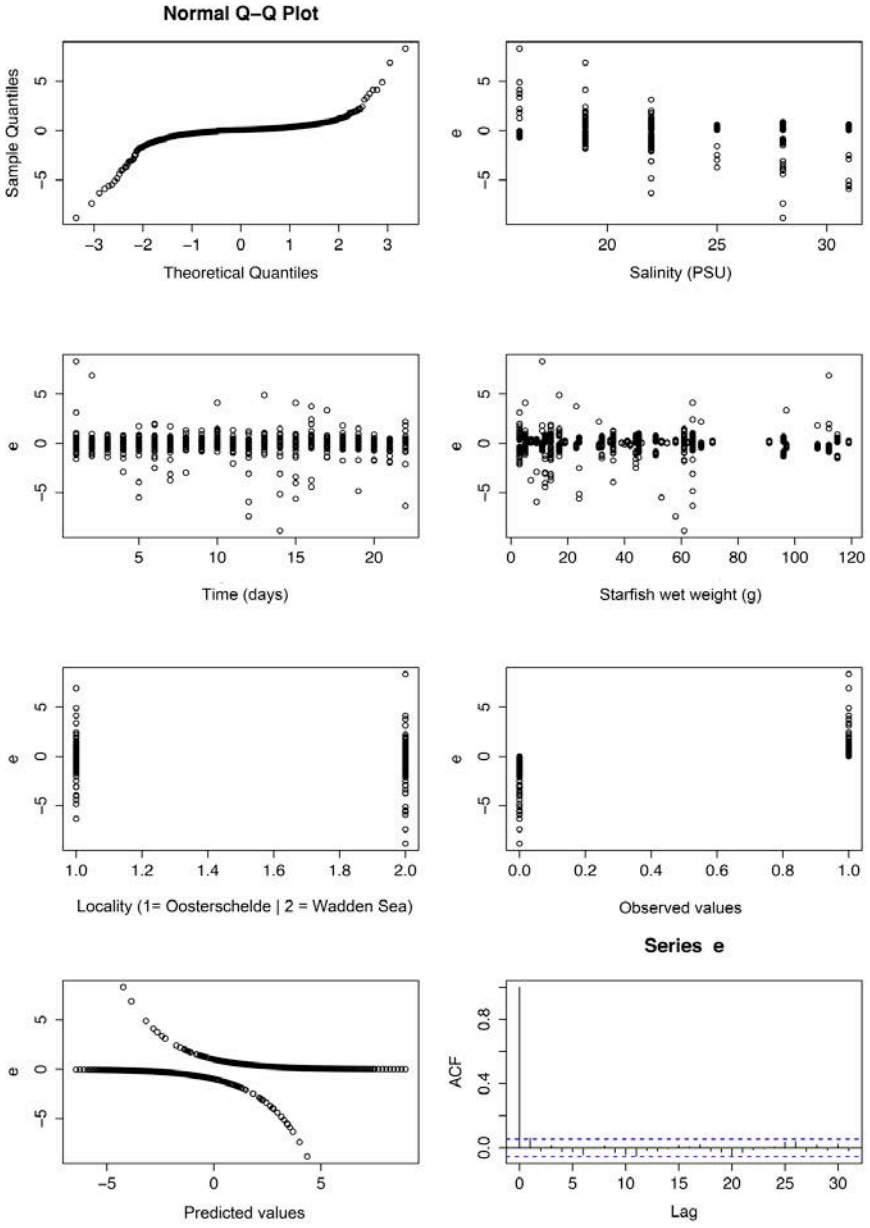
Min	1Q	Median	3Q	Max
-8.8481	-0.1299	0.0627	0.2085	8.3102

	Value	Degrees of freedom
Null deviance	3495.2	155
Residual deviance	1237.8	151

Number of observations: 1301

Number of groups: 60

Residual plots: (e = Pearson's residuals)



Appendix 3.1.4. Linear mixed effects model for mussel size selection

Variables:

LgML	Natural log of mussel length (mm)
Sal	Salinity Treatment
Day	Experimental day
L	Starfish arm length (mm)
Loc	Locality (Wadden Sea or Oosterschelde)
ID	Individual starfish identification number

Linear mixed-effects model fit by REML

Family: Binomial (logit)

AIC	BIC	LogLik
-686.99	-628.06	353.49

Random Effects: Formula: ~1 | ID

	Intercept	Residual
StdDev	0.0717	0.2053

Fixed Effects: LgML ~ Sal + Day + Loc + L + Sal * Day + Day:L + Loc:L

Coefficients:

	Estimate	Std. Error	DF	t-value	p-value
Intercept	2.990	0.1978	2627	15.1182	0
Sal	0.0142	0.0039	51	3.6206	0.0007
Day	0.0247	0.0061	2627	4.0480	0.0001
Loc	-0.2811	0.0935	51	-3.0054	0.0041
L	-0.0015	0.0026	51	-3.0671	0.5712
Sal:Day	-0.0006	0.0002	2627	-3.0671	0.0022
Day:L	-0.0001	0.00004	2627	-2.9994	0.0027
Loc:L	0.0045	0.0017	51	2.6955	0.0095

Correlation:

	Intercept	Sal	Day	Loc	L	Sal:Day	Day :L
Sal	-0.619						
Day	-0.445	0.663					
Loc	-0.790	0.102	-0.006				
L	-0.824	0.118	0.110	0.933			
Sal:Day	0.411	-0.714	-0.912	-0.003	-0.035		
Day:L	0.205	-0.099	-0.492	0.019	-0.203	0.110	
Loc:L	0.721	-0.098	0.004	-0.957	-0.921	0.005	-0.020

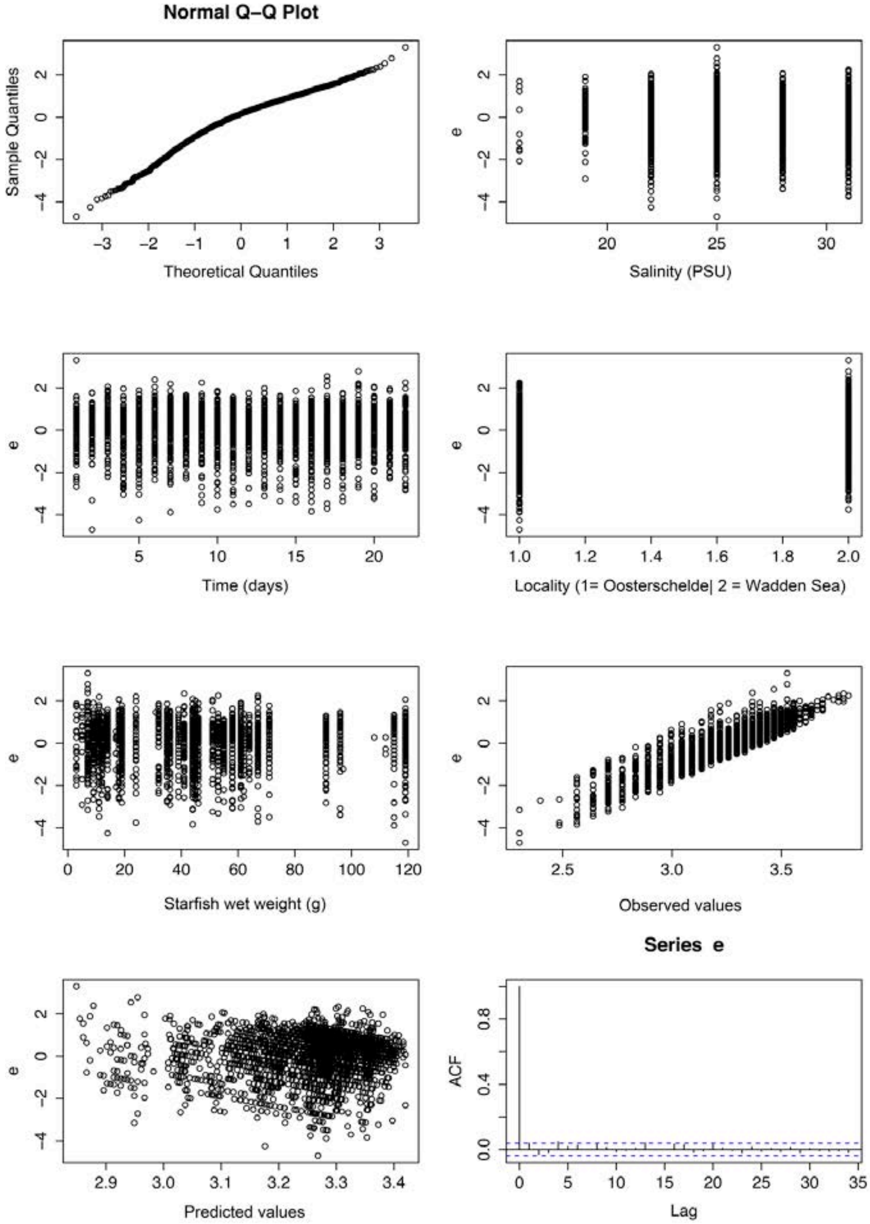
Standardized Within-Group Residuals:

Min	1Q	Median	3Q	Max
-4.6916	-0.5138	0.1632	0.6844	3.2967

Number of observations: 2686

Number of groups: 51

Residual plots: (e = Pearson's residuals)

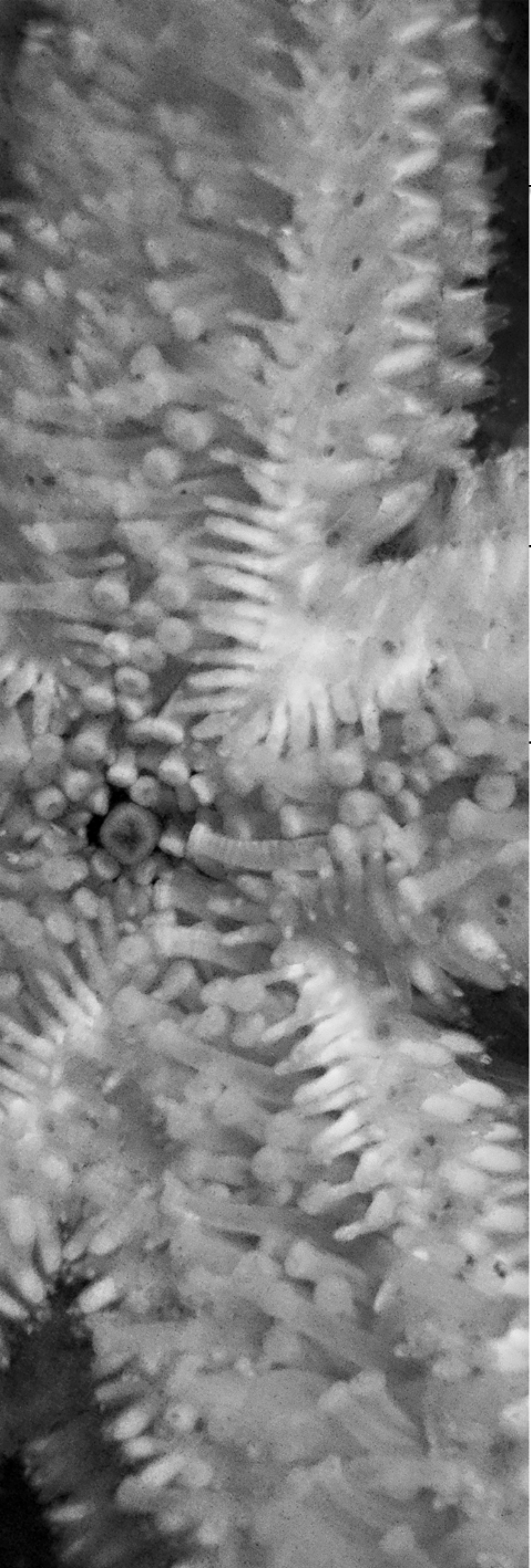


Appendix 3.1.5. Daily feeding rate linear and log-log regression against time

Variables:

FF	Feeding rate as grams mussel flesh per starfish gram
Sal	Salinity Treatment
Day	Experimental day

Best Fit Values						
	31 PSU	28PSU	25PSU	22PSU	19PSU	16 PSU
	linear	*log-log*	*log-log*	*log-log*	*log-log*	*log-log*
Intercept	0.0416	-1.8691	-1.7195	-2.1130	-3.5981	-18.551
Slope	-0.0002	0.2615	0.1784	0.4132	1.322	12.22
Standard Error						
Intercept	0.0035	0.0024	0.0022	0.0057	0.0762	5.6101
Slope	0.0002	-0.0032	0.0021	0.0052	0.0632	4.207
Goodness of Fit						
R square	0.2192	0.3478	0.2918	0.5429	0.6132	0.5735
Slope test						
N	220	220	220	200	200	40
p-value	0.51	0.0001	0.0001	0.0001	0.0001	0.001



Chapter 4

Beyond Food: A foundation species facilitates its own predator

Antonio Agüera
Johan van de Koppel
Jeroen M. Jansen
Tjeerd Bouma
Aad C. Smaal

Published in: Oikos (2015)
doi: 10.1111/oik.01949

Abstract

Facilitation by foundation species can play a critical role in structuring ecological communities. As environmental stress increases, generally more organisms become dependent on the stress buffering provided by foundation species. As such, foundation species may even facilitate their own predators, an interaction that can influence the functioning and structure of the foundation species population and thereby the facilitated organisms. This work presents a case study on the blue mussels and sea stars, where we tested to what extent a foundation species (i.e., blue mussels) may facilitate its own predator (sea star) when exposed to a gradient of environmental stress (hydrodynamic forces). Amelioration of hydrodynamic stress by mussels facilitated sea stars, allowing them to persist on a soft bottom in highly dynamic environment, which would not be possible in the absence of mussels. Moreover, sea stars continue preying on mussels when environmental stress increases. The results suggest that a foundation species may interact with its own predator beyond the role of food source, by ameliorating environmental stress, creating an additional dependence link between the foundation species and the predator, which potentially has major implications for ecosystem structure and stability.

4.1 Introduction

Dominant habitat-forming species, also called foundation species (Dayton 1972), play a critical role in structuring ecological communities. The presence of foundation species often causes changes in the biotic and abiotic components of the ecosystem and thereby has a marked influence on the populations of other organisms. By ameliorating biotic and/or abiotic stress, foundation species facilitate other populations (Bertness and Callaway 1994), including foundation species self-facilitation (Angelini et al. 2011). The magnitude and occurrence of facilitative interactions tend to increase with environmental stress (stress-gradient hypothesis) (Bertness and Callaway 1994, He et al. 2013) so that more species become dependent of the foundation species (Silliman et al. 2011). As whole communities depend upon the habitat conditions that foundation species create (Bertness and Callaway 1994, Stachowicz 2001, Silliman et al. 2011), this facilitation is an important driver of community structuring and functioning (Bruno 2001, Bruno et al 2003, Bulleri 2009).

Foundation species often occupy low trophic levels (Navarrete and Berlow 2006, Altieri et al. 2012) being a reliable food source for predators, that directly benefit from the abundance of their prey (Dankers and Zuidema 1995, Hastings et al. 2007, Hensel and Silliman 2013, Filbee-Dexter and Scheibling 2014, Sanders et al. 2014). Besides feeding on foundation species, some predators may also benefit from habitat modifications by foundation species. As facilitative interactions by foundation species increase in importance with increasing environmental stress (Bertness and Callaway 1994, Silliman et al. 2011, He et al. 2013), species that were only connected through trophic interactions with the foundation species at low stress levels, may be expected to become facilitated at higher stress levels (Burnaford 2004, Silliman et al. 2011). However, the latter remains to be tested as it is still unknown how habitat modification and stress amelioration affect the predators of foundation species. Such understanding is important, as interactions that result in a strong biomass decrease of the foundation species, such as those caused by keystone predators (Paine 1966), may potentially enhance the risk of collapse of the habitat (Scheffer et al. 2001, Folke et al. 2004). Hence we pose the question whether it is possible for a foundation species to facilitate its own predators while still remaining the main prey item, and how this depends on the stress level.

The well recognized combination of *Mytilid* mussel beds on soft substrates as foundation species, with sea stars as keystone predators (Paine 1966, 1974, Menge and Sutherland 1987, Robles et al. 2009) offers a good model to explore under which conditions foundation species can facilitate their direct predators. Mussel aggregations can reduce hydrodynamic stress caused by waves and tidal currents (Folkard and Gascoigne 2009, van Leeuwen et al. 2010) and provide hard substrate and shelter for both sessile and mobile organisms (Buschbaum et al. 2008), thereby acting as foundation species that sustains biodiversity hotspots by facilitating many organisms in soft substrates (Norling and Kautsky 2008). Sea stars of the genus *Asterias* are considered keystone predators, as they have the capacity to exert top-down control on their prey, including mussels (Menge 1982, Witman et al. 2003). This predatory capacity causes important economic losses to the mussel aquaculture industry (Smaal 2002).

Asterias spp. can occur both on soft (Sloan and Robinson 1983) and hard (Guillou et al. 2012) substrate. Sea stars can move and get attached safely on hard substrates (Hennebert et al. 2010), however their locomotion and attachment system is ineffective on non-cohesive soft substrates (Anger et al. 1977). Moreover *Asterias spp.* do not present any special adaptation or behaviour to counteract hydrodynamic stress when inhabiting a soft bottom (Hennebert et al. 2010). Thus, their only mechanism to avoid dislodgement from soft sediments by currents or waves seems to be seeking hydrodynamic sheltering or attaching to hard substrate. Still, *Asterias spp.* are well known inhabitants of subtidal mussel beds (Sloan and Aldridge 1981, Sloan and Robinson 1983, Witman et al. 2003) even when those are located on soft substrate in highly hydrodynamic areas, where they also are important mussel predators (Saier 2001, Agüera et al. 2012). It remains unknown how *Asterias spp.* endure a highly dynamic environment while inhabiting mussel beds on soft bottoms, and to what extent they depend on the capacity of the prey population to ameliorate hydrodynamic stress and provide hard substrate.

We tested the hypothesis that a foundation species can be a food source and simultaneously facilitate a predator by ameliorating environmental stress by studying under what hydrodynamic stress levels each one or both interactions may take place. More specifically, we tested the hypothesis that provision of hard substrate and amelioration of hydrodynamic stress by the foundation *Mytilid* mussel species in soft-bottom habitats allows the sea star predator (of the genus *Asterias*) to persist and continue predating under high hydrodynamic stress levels. We tested this in flume experiments, allowing us to control environmental stress by exposing the system to flow velocity gradients and different substrate types.

4.2 Material & Methods

4.2.1 Flume studies set-up

As a model system we used mussel beds (*Mytilus edulis* L.) as foundation species and the sea star (*Asterias rubens* L.) as predator. *M. edulis* forms extensive beds in the soft substrate all around European estuaries and coastal seas. Some of those areas exhibit a hydrodynamic regime formed by strong tidal currents. For example, in the Wadden Sea, flow velocity changes rapidly with the tides and reaches a maximum speed of over 100 cm s^{-1} (Zimmerman 1976). Mussel beds commonly occur on soft-bottomed areas, where flow velocity has a daily maximum over 50 cm s^{-1}.

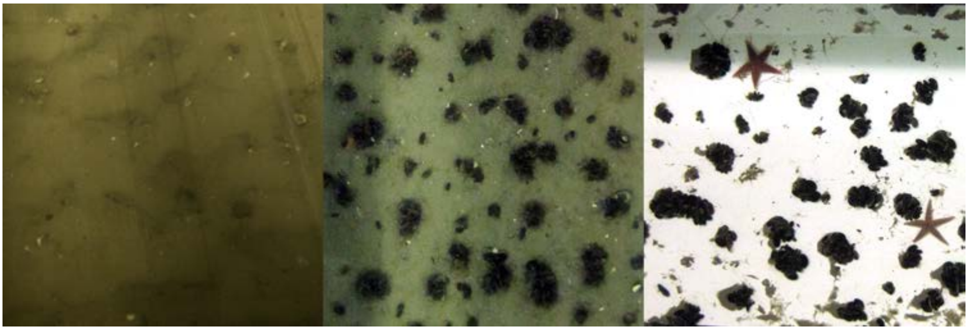


Figure 4.1. Experimental area configurations. From left to right: soft substrate, soft substrate with mussels and hard substrate with mussels.

Flume tank experiments (for flume racetrack description see Bouma et al. 2005) were used to create a subtidal environment. We exposed mussels and sea stars dwelling on both hard and soft substrates to a range of unidirectional flows between 5 and 50 cm s^{-1}. The experimental area within the flume was restricted to an area of 1.80 by 0.6 m (1,08 m^2). The same area was prepared with different system configurations (Figure 4.1): soft substrate, soft substrate with mussels and hard substrate with mussels. Soft substrate consisted of a 10 cm thick layer of natural sediment obtained from the Oosterschelde estuary (The Netherlands), a mix of sand and silt with a minor proportion of shell fragments. Hard substrate consisted of a plate of trespa® which was sanded down to increase surface roughness. Where used, mussels were always present at a density of 1000 g m^{-2}. Mussels were added to the system at least 24 hours before the experiment to allow for acclimation and to allow them to attach in their typical aggregations (Buschbaum et al. 2008).

Both mussels and sea stars used in flume experiments were obtained from soft substrates bottom culture plots in the Oosterschelde estuary. Mussels (5.6 to 31 mm length) were captured using a small dredge from a boat and sea stars (41.3 to 55.2 mm arm length) were captured by the same small dredge and by hand. All animals were kept in a recirculation system tank set inside the same room containing the flume tank, maintaining all animals under the same conditions of salinity, pH and temperature than those of the flume. When individuals were transferred to the flume for the experiments sea stars were transported in small buckets avoiding emersion and reducing handling to a minimum.

4.2.2 Sea star dislodgement and reaction to continuously increasing flow velocity

These experiments aimed to quantify the response of sea stars to a continuous increase in the flow velocity, assessing the flow speed at which sea stars stop moving or get dislodged from the substrate. Different experimental treatments allowed us to assess the contribution of aggregated mussels relative to the different type of substrates mentioned in the previous paragraph. In the experiments flow velocity was increased from 5 to 50 cm s^{-1} by increasing the flume flow velocity by 5 cm s^{-1} every 15 minutes, roughly mimicking the velocity change that may be experienced over a tide. Three different system configurations were used: soft substrate, soft substrate with mussels and hard substrate with mussels. For each system configuration 5 trials were performed. In each trail ten new sea stars, were used. Sea stars were added to the experiment during a period with no water current for 15 minutes, after which the experiment started.

During the 15 minutes interval at each flow velocity, animals were observed for movement. Dislodged animals were removed from the flume; arm length was measured down to the nearest millimetre, wet weight was measured to the nearest gram and the flow at which they were dislodged was noted. A sea star was considered dislodged when it was pulled off the substrate and pushed outside the experimental area. At the end of each trial remaining sea star were also measured. Data resulting from these experiments was binary (0,1). At each flow velocity step dislodged animals were given a 1, while animals that moved to next flow step were given a 0. Additionally when an individual was observed moving at any point during the 15 minutes of each flow step a 1 was noted, size of moving animals was not recorded to not interfere with their attempts to counteract flow. The role of flow, substrate, presence of mussels and individual sea star size in dislodgement was analysed by means of general linear models with binomial distributions. For movement only the flow, substrate and presence of mussels was used in the models.

4.2.3 Effects of constant flow on sea star food searching and feeding behaviour

This experiment consisted of exposures to a constant flow velocity for 48 hours and aimed to describe the activity of sea stars when exposed constantly to different flow velocities. Measurements included feeding and time spent moving (as moving from one location to another within the experimental area, movements without changing location were not considered). For these experiments only the system configurations of soft substrate with mussels and hard substrate with mussels were used. Flow velocities used were 10, 20, 30, 40 and 50 cm s^{-1}. In total 10 trials were performed; 5 with soft substrate and another 5 with hard substrate (one at each flow velocity). In each trial 10 sea stars were used, resulting in a density of 300 g m^{-2}.

For these experiments a new mussel bed was laid before each trial. Mussels were set in the system 24 hours before starting the trial. The flume was started and set at a flow velocity of 5 cm s^{-1}, sea stars were added to the system at that velocity. Flow velocity was increased at a rate of 5 cm s^{-1} every 5 minutes until the designated flow velocity was reached. Sea stars were then left to feed and move during 48 hours under the designated flow velocity. Those experiments were performed in 24 hour light and a camera was set to record a picture of the experimental area every 30 seconds.

Using time-lapse pictures, percentage of animals that were observed feeding at anytime during the experiment was calculated, feeding animals can be distinguished clearly in the pictures by the typical humped position adopted by sea stars when embracing a prey. At the end of each trial eaten mussel shells were recovered, counted and measured. The total weight of consumed mussels was assessed by approximating weight from mussel shell size using a regression of mussel weight against mussel shell length using the mussels in stock for the experiments. Additionally percentage of time spent moving by each individual was also assessed. Feeding observations resulted in binomial data (1,0) and the role of flow and substrate was analyzed using general lineal models with binomial distribution. Time spent moving was analyzed by log regression. Best fit was selected by R-squared values. And the model was validated by residual analysis.

All data analysis was performed using R (v. 2.15.2) (R Core Team in press). Model selection of the general linear models with binomial distributions models was done considering all the variables available in each case (flow, sea star size, system configuration, etc.). Best fit was selected by comparison of Akaike Information Criterion (AIC) values. Model validation was performed with residuals plots following (Zuur et al. 2009) recommendations. Final model details and validation plots are presented in Appendix 3.1

4.3 Results

4.3.1 Sea star dislodgement and reaction to continuously increasing flow velocity

Sea stars stopped moving when reaching certain flow velocity (Figure 4.2), suggesting that keeping still is a first reaction to short-term changes in hydrodynamic stress. Constantly increasing flow reduced *A. rubens* movements (Figure 4.2). In trials with mussels on a hard substrate, almost all individuals kept moving until flow velocity reached 30 cm s^{-1} with half of them stopping when a flow velocity of 42 cm s^{-1} was reached. This response was, however, much stronger for *A. rubens* on soft substrate with mussels: increasing flow velocity immediately resulted in a decrease of animals moving. At a flow velocity of 19 cm s^{-1} half of the animals stopped moving and less than 10% kept moving when flow velocity reached 35 cm s^{-1}. When mussels were not present on soft substrate half the sea stars stopped moving at a flow velocity of 16 cm s^{-1}, which did not significantly differ from the flow velocity observed for soft substrate with mussels (See Appendix 4.1 for details).

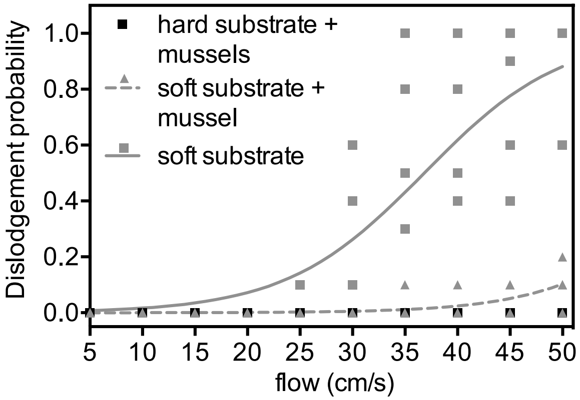


Figure 4.2. Sea star dislodgement probability under constantly increasing flow velocity as a function of flow velocity, substrate type and presence or not of mussels. Data points represent percentage of dislodged sea stars observed at each flow during the experiments. Lines are general linear model with binomial data distribution output for the different system configurations (see appendix I for parameters and details). The probability of dislodgment is zero when sea stars are on hard substrate. In trials with soft substrate, the probability of sea stars being dislodged increased with flow velocity, however mussels had a significant effect ($p\text{-value} < 0.01$) reducing dislodgment of sea stars at high flows.

Increasing flow increased the probability of animals being dislodged with significant differences between system configurations (Figure 4.3). No dislodgement was observed in trials with hard substrate. In contrast, the presence of mussels had a significant effect in the dislodgment of sea stars observed in soft substrate trials. Increasing flow velocity resulted in an increase of the probability of sea stars being dislodged from the soft substrate when mussels were absent. As such, some individuals got dislodged from the substrate at a flow of 25 cm s^{-1} with half of them being dislodged when reaching 35 cm s^{-1}. However, when mussels were present at the soft substrate, no individual was dislodged below 35 cm s^{-1} and only 10% were dislodged at 50 cm s^{-1} (See Appendix 4.1 for details).

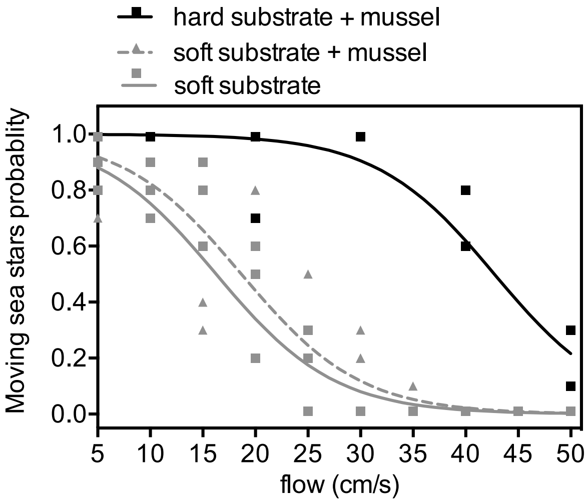


Figure 4.3. Sea star movement at flow velocity. Probability of observing a moving sea star at flow velocity when this was being constantly increased. Data points represent percentage of sea stars observed moving at flow during the experiments. Lines are general linear model with binomial data distribution output for the different system configurations (see appendix I for parameters and details). Flow velocity significantly reduced the number of sea stars moving (p-value < 0.01), however there is a significant difference among the substrate types (p-value <0.01) with sea stars stopping moving at lower flows when on soft substrate. No significant difference related to the presence of mussels on soft substrate was observed (p-value = 0.058).

4.3.2 Effects of constant flow in sea star behaviour

Sea stars stopped moving when reaching certain flow speed (Figure 4.3), showing that keeping still is a first reaction to short-term changes in hydrodynamic stress. Experiments with a longer-time exposure (i.e., up to 48 hours) to constant flow showed that sea stars can actively move (i.e., searching for food) at all flow velocities, however activity decreased at higher flows (Figure 4.4). Activity, measured as the proportion of time spent moving, clearly showed that sea stars spent less time moving at higher flows. On hard substrate, sea stars reduced the time they spent moving by half when flow velocity reached 20 cm s^{-1}. On soft substrate we observed a similar effect with sea stars reducing the time they spent moving by half at a flow velocity of 20 cm s^{-1}. However sea stars on soft substrate spent significantly less time searching at all flow velocities. Difference between soft and hard substrate became smaller as flow velocity increased (Figure 4.4) (See Appendix 4.1 for details).

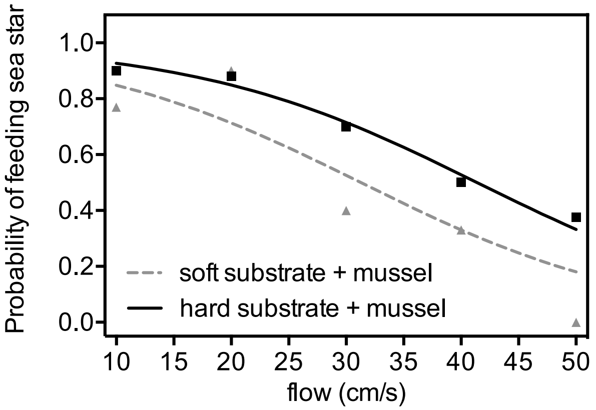


Figure 4.4. Feeding activity. Percentage of sea stars that where observed feeding at during the 48 hours trials as function of flow and substrate type. Lines are general linear model with binomial data distribution output for the different system configurations (see appendix I for parameters and details). Feeding activity decreased with flow (p-value < 0.01). When sea stars were on soft substrate activity was significantly lower (p-value = 0.0176).

Flow velocity had an effect on sea star predation rate. As flow increases, fewer animals fed during the experiments, and the probability of observing a sea star feeding decreased (Figure 4.5). This effect results from changes on sea star's feeding frequency and is directly related to predation rate (Agüera et al. 2012). Substrate had a significant effect on feeding frequency. On hard substrate half of the sea stars did not feed during the 48 hours trial at flow velocity of 40 cm s^{-1}. On soft substrate half the sea stars stopped feeding when flow velocity was 30 cm s^{-1}. (See Appendix 4.1 for details). Besides de reduced feeding activity sea stars were able to consume a substantial amount of mussels. On hard substrate sea stars consumed up to 14% of the available mussel biomass during the experiments when flow velocity was below 20 cm s^{-1}, this amount decreased to 7% at a flow velocity of 30 cm s^{-1} with little more than 1% being consumed in the 50 cm s^{-1} trial. On soft substrate the pattern observed was similar, sea stars removed up to 10% of available mussels at flow velocities below 20 cm s^{-1}, feeding decreased to 5% by 30 cm s^{-1}, with barely over 2% of mussel biomass being consumed at 40 cm s^{-1}.

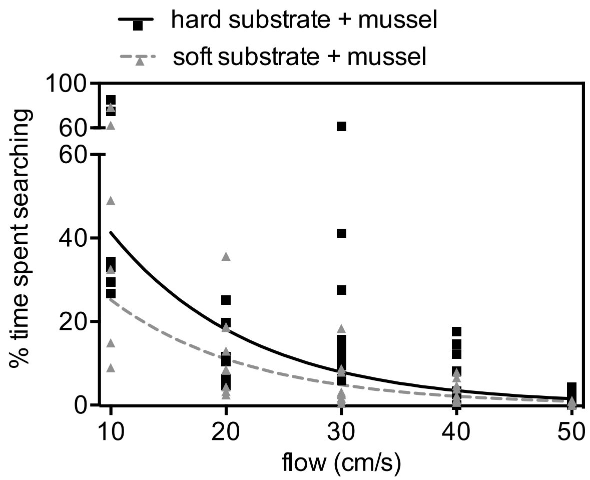


Figure 4.5. Percentage of time sea stars spent searching (moving) when exposed to a constant flow during 48 hours as a function of flow velocity. Data represent individual sea star fraction of time spent searching from all 48 hour trials. Lines are linear regression output (see appendix I for parameters and details). The time sea stars spent searching decreased with flow (p-value < 0.01) independently of substrate nature, however in the trials using soft substrate searching time was significantly lower (p-value = 0.048).

4.4 Discussion

Knowledge on the interactions of foundation species with the organisms they facilitate is fundamental to our understanding of community structure and functioning. Facilitation is still poorly understood in the case where the facilitated organism is a predator of the foundation species. Differentiating between the net effects of trophic interactions and facilitation due to habitat modifications is difficult (Sanders and van Veen 2011), however our study shows that a predator may interact with its foundation prey beyond just preying on it. Following the stress-gradient hypothesis (Bertness and Callaway 1994, Holmgren and Scheffer 2010) facilitation of a predator by a foundation prey species predominantly takes place when environmental stress reaches sufficient importance. Facilitation depends on environmental settings and its importance increases with environmental stress with both trophic and facilitative interactions simultaneously taking place under intermediate stress levels (Figure 6). The generality of the schematization needs further testing on other combinations of foundation and predator species. Facilitation of a predator by its prey can have major consequences for the stability of the foundation species population, potentially increasing the risk of collapse of the habitat that depends on the foundation species.

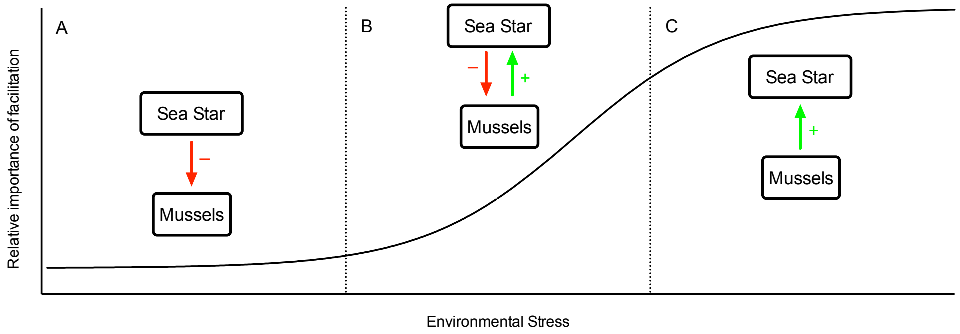


Figure 4.6. Schematic graph showing the transition from a trophic interaction to facilitation with environmental stress between mussel and sea stars. A low stress levels, typical keystone example (Paine 1966) where predator top-down control a dominant species; B intermediate levels of stress (this study), the dominant species (foundation species) ameliorate environmental stress and facilitates the predator that can still feed on the foundation species; C high levels of stress that limits predation completely (Silliman et al. 2011), the predator is still being facilitated but remaining stress is large enough to limit the access of the predator to the foundation species.

4.4.1 The temporal distribution of environmental stress: tidal cycles

Positive interactions tend to be more common and important in physically stressful habitats than in benign habitats (Bertness and Callaway 1994), a direct consequence of the capacity of foundation species to reduce physical stresses that may limit the distribution of other species. Stress amelioration by the foundation mussel species allows sea stars to persist in the habitat, and continue predating substantial amounts of mussels. However in many habitats, stress is not constant in time. Depending on the level of environmental stress, the importance of the facilitation and the trophic interaction may alternate: facilitation dominates at high levels of stress and predation takes place at lower levels, as stress gradient theory predicts. In our specific case, such alternation occurs within a single tidal cycle (Figure 4.7), which produces changes in tidal flow direction and velocity. This illustrates that the transition from trophic interaction to facilitation may be frequently occurring and may be induced by short-term variations in environmental conditions. Even though facilitation only takes places at specific moments in time, its effects persist for longer periods as it allows a predator to persist. This re-emphasizes the well recognized importance of facilitation for the community functioning and composition (Bulleri 2009, Butterfield 2009, Silliman et al. 2011).

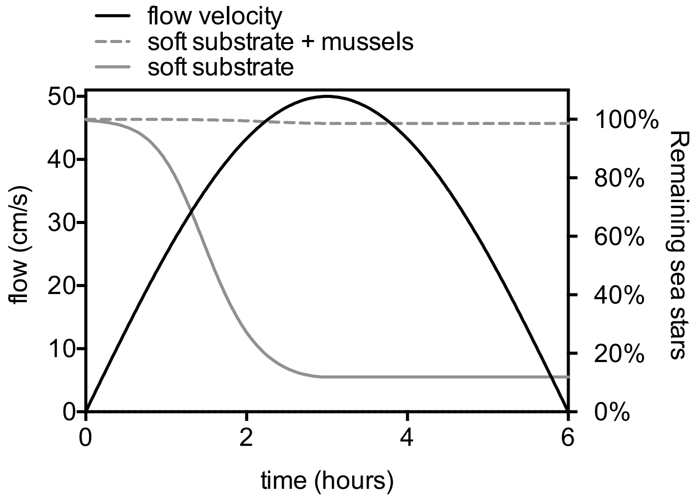


Figure 4.6. Effect of flow on the sea star population, inhabiting on soft substrate, during one tide displacement. Maximum flow considered is 50 cm s^{-1}. In tidal movements flow velocity goes from slack time (low flow velocity) at high or low tide to fast flows when the water level is changing. When mussels are not present the change in flows between two consecutive slack times removes more than 80% of the sea star population, however when mussels are present, the sea star population is not affected by the changes in flow velocity, remaining in the mussel bed.

4.4.2 Negative feedback and ecosystem stability

The effects of foundation species in the community have been the object of many recent studies (Rohr et al. 2009, Angelini et al. 2011, Silliman et al. 2011). Despite this, feedbacks on the foundation species from the facilitated species are still not so well understood, though this may play a key role in community structuring (Jones et al. 1997). Facilitating your own predator has an obvious negative effect on the foundation species population and could compromise the stability of the created habitat. Foundation species often need to persist above a critical density or biomass, in order to sustain the created habitat (Rietkerk et al. 2004, Bouma et al. 2009). Facilitation creates another link between predator and prey. This dependence of the predator on the foundation species may also contribute to swarming events and/or formation of feeding fronts. Traditionally swarming events and the formation of feeding fronts have been mainly associated to different trophic mechanisms (e.g. prey abundance, absence of top predators) (Silliman et al. 2013) that resulted in predators attaining high densities around prey patches. However the facilitative interaction found in this study may also have a role in such concentration of predators, as it reduces predator mortality and/or increases the resilience of the predator in the created habitat. In the end facilitation may allow this predator to significantly reduce foundation species biomass increasing the risk of collapsing of the foundation species population and the created habitat, thereby jeopardizing and affecting the whole community, including its own population. It is possible for the system to remain stable when either (1) the remaining environmental stress is able to reduce predator performance reducing its predation impact (Bruno et al. 2003, Silliman et al. 2011) or when (2) the facilitated predator does not feed exclusively or massively on the foundation species (Burnaford 2004). However, both situations are apparently not the case in our model system.

Subtidal mussel bed stability can be largely compromised by sea star predation even in soft substrate areas under high speed currents (Saier 2001, Agüera et al. 2012). Under constant hydrodynamic stress sea stars were able to consume substantial amounts of mussels. In the field, where hydrodynamics stress is not constant, but switching from low to high levels and back, it is expected that sea star predation is higher. Moreover, higher mussel density has a higher effect ameliorating hydrodynamic stress (Folkard and Gascoigne 2009) meaning that in more dense mussels beds predation may also be higher.

We conclude that predatory interactions and facilitation are not mutually exclusive as they can occur simultaneously. Following the stress gradient hypothesis, the importance of this facilitative interaction increases with stress. Mobile predators suffer the remaining stress resulting after amelioration by the foundation species and predation is reduced, yet still the decrease in predation capacity will be less that it would be expected without facilitation (Bruno et al. 2003), as predators are able to persist and have access to the prey thanks to this facilitation. Moreover this facilitation may result in an increase of the predator population that depends on its prey not only for food, but also for shelter. As a consequence the increase of ecosystem stability with environmental stress predicted by the stress gradient hypothesis (Bertness et al. 1999) is put into question. The facilitation of predators is important to understand the role of facilitation in ecosystem functioning and structuring in stressful environments. We have shown here that the facilitation of predators by foundation species may not only have a direct impact on the foundation species populations, but also their role as a foundation species and how they affect environmental stress and thus, ecosystem stability.

References

Agüera, A., M. Trommelen, F. Burrows, J. M. Jansen, T. Schellekens, and A. Smaal. 2012. Winter feeding activity of the common starfish (*Asterias rubens* L.): The role of temperature and shading. Journal of Sea Research 72:106–112.

Altieri, A. H., M. D. Bertness, T. C. Coverdale, N. C. Herrman, and C. Angelini. 2012. A trophic cascade triggers collapse of a salt-marsh ecosystem with intensive recreational fishing. Ecology 93:1402–1410.

Angelini, C., A. H. Altieri, B. R. Silliman, and M. D. Bertness. 2011. Interactions among Foundation Species and Their Consequences for Community Organization, Biodiversity, and Conservation. BioScience 61:782–789.

Anger, K., U. Rogal, G. Schriever, and C. Valentin. 1977. In-situ investigations on the echinoderm *Asterias rubens* as a predator of soft-bottom communities in the western Baltic Sea. Helgoländer Wissenschaftliche Meeresuntersuchungen 29:439–459.

Bertness, M. D., and R. M. Callaway. 1994. Positive interactions in communities. Trends in Ecology and Evolution 9:191–193.

Bertness, M. D., G. H. Leonard, J. M. Levine, P. R. Schmidt, and A. O. Ingraham. 1999. Testing The Relative Contribution Of Positive And Negative Interactions In Rocky Intertidal Communities. Ecology 80:2711–2726.

Bouma, T. J., M. B. De Vries, E. Low, G. Peralta, I. C. Tánczos, J. van de Koppel, and P. M. J. Herman. 2005. Trade-offs related to ecosystem engineering: a case study on stiffness of emerging macrophytes. Ecology 86:2187–2199.

Bouma, T. J., S. Olenin, K. Reise, and T. Ysebaert. 2009. Ecosystem engineering and biodiversity in coastal sediments: posing hypotheses. Helgoland Marine Research 63:95–106.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution 18:119–125.

Bulleri, F. 2009. Facilitation research in marine systems: state of the art, emerging patterns and insights for future developments. Journal of Ecology 97:1121–1130.

Burnaford, J. L. 2004. Habitat modification and refuge from sublethal stress drive a marine plant-herbivore association. Ecology 85:2837–2849.

Buschbaum, C., S. Dittmann, J.-S. Hong, I.-S. Hwang, M. Strasser, M. Thiel, N. Valdivia, S.-P. Yoon, and K. Reise. 2008. Mytilid mussels: global habitat engineers in coastal sediments. Helgoland Marine Research 63:47–58.

Butterfield, B. J. 2009. Effects of facilitation on community stability and dynamics: synthesis and future directions. Journal of Ecology 97:1192–1201.

Dankers, N., and D. R. Zuidema. 1995. The Role of the Mussel (*Mytilus edulis* L.) and Mussel Culture in the Dutch Wadden Sea. Estuaries 18:71–71.

Dayton, P. K. 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. Pages 81–96 *in*. … conservation problems in Antarctica, Kansas.

Filbee-Dexter, K., and R. E. Scheibling. 2014. Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. Marine Ecology Progress Series 495:1–25.

Folkard, A. M., and J. C. Gascoigne. 2009. Hydrodynamics of discontinuous mussel beds: Laboratory flume simulations. Journal of Sea Research 62:250–257.

Folke, C., S. Carpenter, B. Walker, M. Scheffer, T. Elmqvist, L. Gunderson, and C. S. Holling. 2004. Regime Shifts, Resilience, and Biodiversity in Ecosystem Management. Annual Review of Ecology, Evolution, and Systematics 35:557–581.

Guillou, M., G. Joly-Turquin, S. Leyzour, P. Pernet, and P. Dubois. 2012. Factors controlling juvenile growth and population structure of the starfish Asterias rubens in intertidal habitats: field and experimental approaches. Journal Of The Marine Biological Association Of The United Kingdom 92:367–378.

Hastings, A., J. E. Byers, J. A. Crooks, K. Cuddington, C. G. Jones, J. G. Lambrinos, T. S. Talley, and W. G. Wilson. 2007. Ecosystem engineering in space and time. Ecology Letters 10:153–164.

He, Q., M. D. Bertness, and A. H. Altieri. 2013. Global shifts towards positive species interactions with increasing environmental stress. Ecology Letters 16:695–706.

Hennebert, E., D. Haesaerts, P. Dubois, and P. Flammang. 2010. Evaluation of the different forces brought into play during tube foot activities in sea stars. The Journal of Experimental Biology 213:1162–1174.

Hensel, M., and B. R. Silliman. 2013. Consumer diversity across kingdoms supports multiple functions in a coastal ecosystem. Proceedings of the National Academy of Sciences of the United States of America 110:20621–20626.

Holmgren, M., and M. Scheffer. 2010. Strong facilitation in mild environments: the stress gradient hypothesis revisited. Journal of Ecology 98:1269–1275.

Jones, C. G., J. H. Lawton, and M. Shachak. 1997. Positive and Negative Effects of Organisms As Physical Ecosystem Engineers. Ecology 78:1946–1957.

Menge, B. A. 1982. Effects of feeding on the environment: Asteroidea. Pages 521–551 *in* M. Jangoux, J. M. Lawrence, and J. M. Lawrence, editors. Echinoderm Nutrition. A.A. Balkema, Rotterdam.

Menge, B. A., and J. P. Sutherland. 1987. Community Regulation: Variation in Disturbance, Competition, and Predation in Relation to Environmental Stress and Recruitment. The American Naturalist 130:730–757.

Navarrete, S. A., and E. L. Berlow. 2006. Variable interaction strengths stabilize marine community pattern. Ecology Letters 9:526–536.

Norling, P., and N. Kautsky. 2008. Patchy mussel (Mytilus edulis) communities as islands of high biodiversity in subtidal sediment habitats in the Baltic Sea. Aquatic Biology 4:75–87.

Paine, R. T. 1966. Food web complexity and species diversity. The American Naturalist 100:65–75.

Paine, R. T. 1974. Intertidal community structure. Oecologia 15:93–120.

Rietkerk, M., S. C. Dekker, P. C. de Ruiter, and J. van de Koppel. 2004. Self-Organized Patchiness and Catastrophic Shifts in Ecosystems. Science 305:1926–1929.

Robles, C. D., R. A. Desharnais, C. Garza, M. J. Donahue, and C. A. Martinez. 2009. Complex equilibria in the maintenance of boundaries: experiments with mussel beds. Ecology 90:985–995.

Rohr, J. R., J. R. Rohr, C. G. Mahan, C. G. Mahan, and K. C. Kim. 2009. Response of arthropod biodiversity to foundation species declines: The case of the eastern hemlock. Forest ecology and management 258:1503–1510.

Saier, B. 2001. Direct and indirect effects of seastars *Asterias rubens* on mussel beds (*Mytilus edulis*) in the Wadden Sea. Journal of Sea Research 46:29–42.

Sanders, D., and F. J. F. van Veen. 2011. Ecosystem engineering and predation: the multi-trophic impact of two ant species. Journal of Animal Ecology 80:569–576.

Sanders, D., C. G. Jones, E. Thébault, T. J. Bouma, T. van der Heide, J. van Belzen, and S. Barot. 2014. Integrating ecosystem engineering and food webs. Oikos 123:513–524.

Scheffer, M., S. R. Carpenter, J. A. Foley, B. Walker, and C. Folke. 2001. Catastrophic shifts in ecosystems. Nature 413:591–596.

Silliman, B. R., M. W. McCoy, C. Angelini, R. D. Holt, J. N. Griffin, and J. van de Koppel. 2013. Consumer Fronts, Global Change, and Runaway Collapse in Ecosystems. Annual Review of Ecology, Evolution, and Systematics 44:503–538.

Silliman, B. R., Silliman, B. R., M. D. Bertness, A. H. Altieri, J. N. Griffin, M. C. Bazterrica, F. J. Hidalgo, C. M. Crain, and M. V. Reyna. 2011. Whole-Community Facilitation Regulates Biodiversity on Patagonian Rocky Shores. PLoS ONE 6:e24502.

Sloan, N. A., and S. M. C. Robinson. 1983. Winter feeding by asteroids on a subtidal sandbed in British Columbia. Ophelia 22:125–140.

Sloan, N. A., and T. H. Aldridge. 1981. Observations on an aggregation of the starfish *Asterias rubens* L. in Morecambe Bay, Lancashire, England. Journal of Natural History 15:407–418.

Smaal, A. C. 2002. European mussel cultivation along the Atlantic coast : production status , problems and perspectives. Hydrobiologia 484:89–98.

Stachowicz, J. J. 2001. Mutualism, Facilitation, and the Structure of Ecological Communities. BioScience 51:235–246.

van Leeuwen, B., D. C. M. Augustijn, B. K. van Wesenbeeck, S. J. M. H. Hulscher, and M. B. De Vries. 2010. Modeling the influence of a young mussel bed on fine sediment dynamics on an intertidal flat in the Wadden Sea. Ecological Engineering 36:145–153.

Witman, J. D., S. J. Genovese, J. F. Bruno, J. W. McLaughlin, and B. I. Pavlin. 2003. Massive Prey Recruitment and the Control of Rocky Subtidal Communities on Large Spatial Scales. Ecological Monographs 73:441–462.

Zimmerman, J. T. F. 1976. Mixing and flushing of tidal embayments in the western Dutch Wadden Sea. Part I: Distribution of Salinity and calculation of mixing time scales. Netherlands Journal of Sea Research 10:149–191.

Zuur, A. F., E. N. Ieno, A. A. Savelier, and G. M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R (statistics for Biology and Health). Pages 574–574. Springer, Berlin.

Appendix 4.1 Statistical model parameters and residual plots

Appendix 4.1.1 General linear model for starfish dislodgement.

Variables:

flow	Flow velocity in cm • s^{-1}
factor	Mussels present or not.

General Linear Mixed model with binomial distribution (only soft substrate)

AIC: 364.9

Deviance results

Min	1Q	Median	3Q	Max
-2.0635	-0.3232	-0.1259	-0.0229	2.9854

Coefficients:

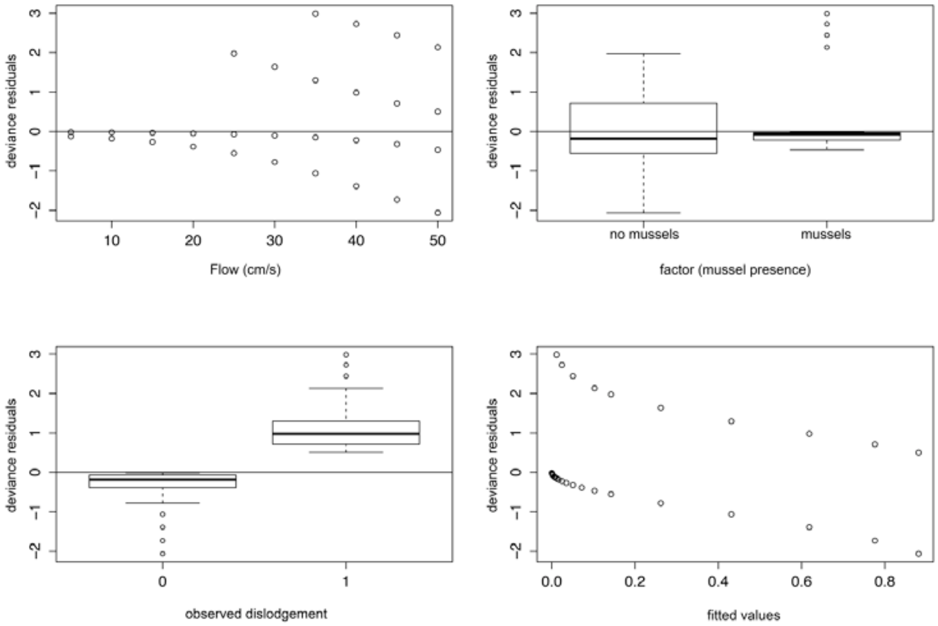
	Estimate	Std. Error	z value	p-value
Intercept	-5.5936	0.5409	-10.342	0
flow	0.1519	0.0148	10.262	0
factor	-4.1684	0.4211	-9.898	0

Dispersion parameter for Binomial family taken to be 1

	Value	Degrees of freedom
Null deviance	728.8	789
Residual deviance	358.9	787

Number of Fisher Scoring iterations: 7

Residual plots



Appendix 4.1.2 General linear model for starfish reaction to continuously increasing flow velocity.

Variables:

flow	Flow velocity in cm • s^{-1}
factor	Factor[1]: soft substrate with mussels; Factor[2]: hard substrate with mussels. Reference factor is soft substrate without mussels.

General Linear Mixed model with binomial distribution

AIC: 590.8

Deviance results

Min	1Q	Median	3Q	Max
-2.8084	-0.5436	-0.1144	0.5661	2.4310

Coefficients:

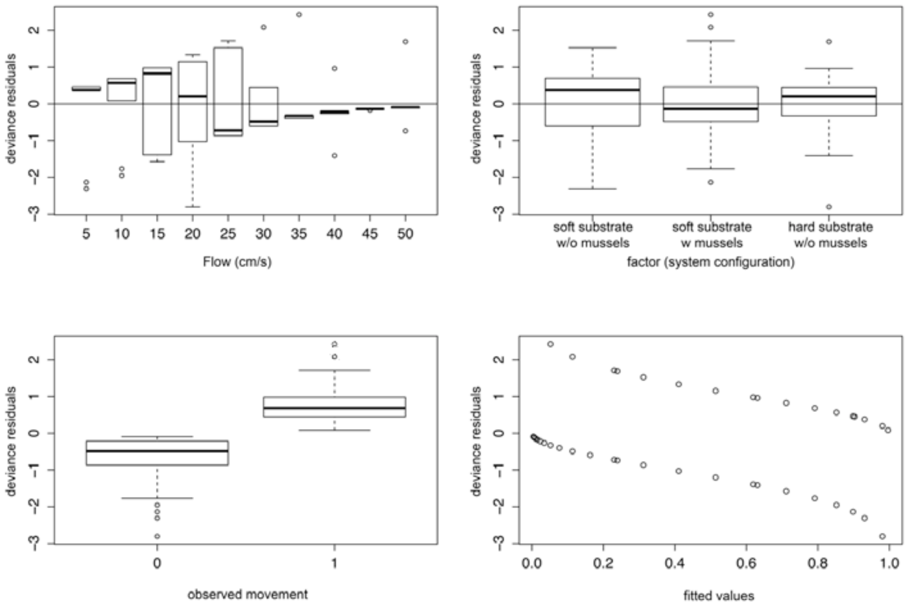
	Estimate	Std. Error	z value	p-value
Intercept	-3.4436	0.2874	11.980	0
flow	-0.1693	0.0124	-13.696	0
Factor[1]	-0.4168	0.2201	-1.893	0.0583
Factor[2]	3.8678	0.4381	8.827	0

Dispersion parameter for Binomial family taken to be 1

	Value	Degrees of freedom
Null deviance	1048	762
Residual deviance	582.8	759

Number of Fisher Scoring iterations: 6

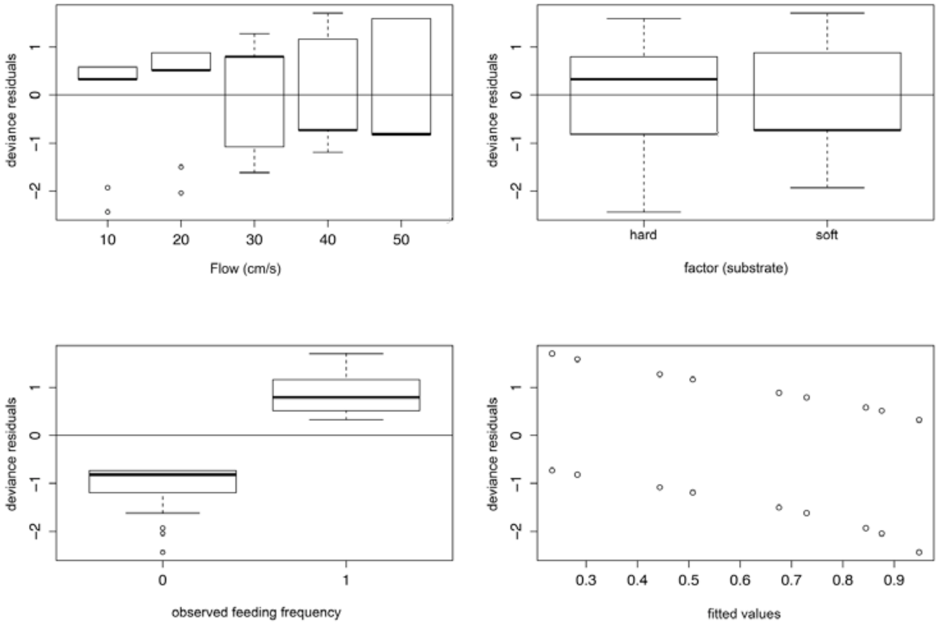
Residual plots



Appendix 4.1.3 General linear model feeding frequency general linear model with binomial distribution:

Variables:				
flow	Flow velocity in cm • s^{-1}			
factor	Substrate: soft or hard			
General Linear Mixed model with binomial distribution				
AIC: 105.57				
Deviance results				
Min	1Q	Median	3Q	Max
-2.4353	-0.7942	0.3253	0.8629	1.7051
Coefficients:				
	Estimate	Std. Error	z value	p-value
Intercept	3.8729	0.8635	4.485	0
flow	-0.0960	0.0224	-4.293	0
factor	-1.2184	0.5133	-2.374	0.0176
Dispersion parameter for Binomial family taken to be 1				
		Value		Degrees of freedom
Null deviance		127.57		93
Residual deviance		99.57		91
Number of Fisher Scoring iterations: 4				

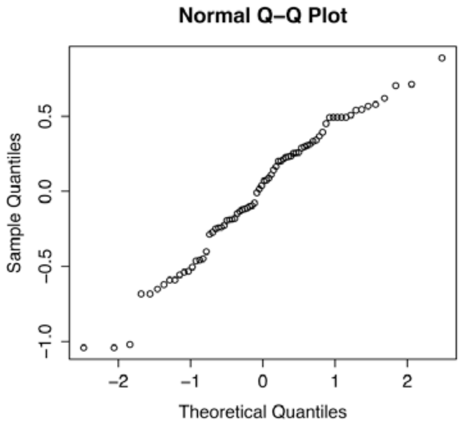
Residual plots

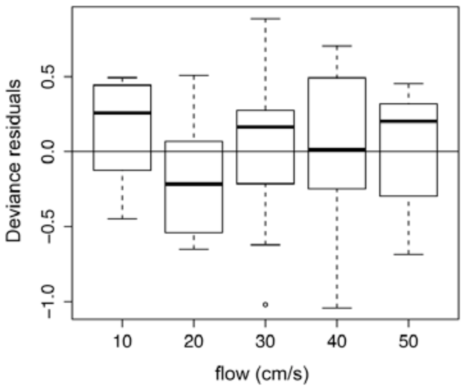


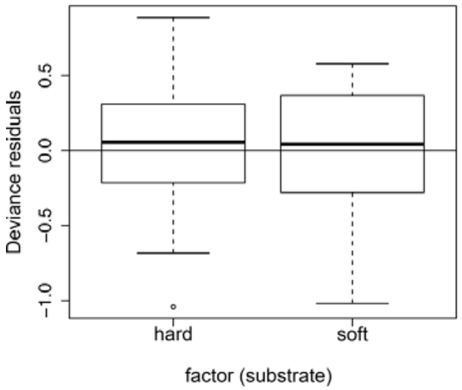
Appendix 4.1.4 Linear model for time spent searching

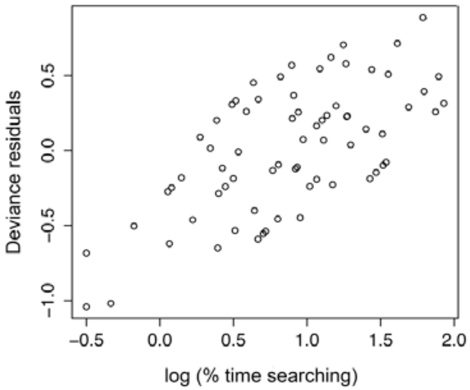
Variables:				
lgS	Percentage of time spent searching prey			
flow	Flow velocity in cm • s^{-1}			
factor	Substrate: soft or hard			
Linear Model: lgS ~ flow + factor (substrate)				
R-squared: 0.484		F-statistic: 36.15 with 2 and 73DF		p-value = 0
Deviance results				
Min	1Q	Median	3Q	Max
-1.0418	-0.2548	0.05355	0.3184	0.8874
Coefficients:				
	Estimate	Std. Error	t value	p-value
Intercept	1.9741	0.1458	13.531	0
flow	-0.0358	0.0042	-8.502	0
factor	-0.2142	0.1056	-2.028	0.0462
		Value	Degrees of freedom	
Residual deviance		0.4496	73	

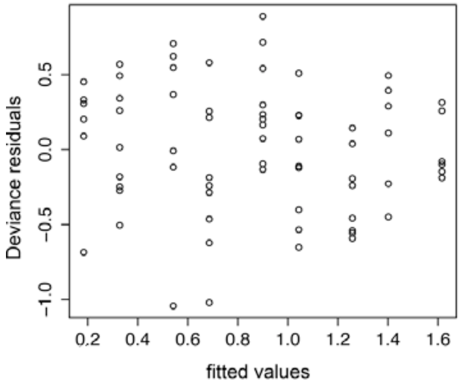
Residual plots (residuals are Pearson's type)











Chapter 5

Mussel association affects prey size selection by starfish

Antonio Agüera
Tim Schellekens
Jeroen M. Jansen
Aad C. Smaal

Submitted for publication

Abstract

Predator prey selection is of interest to community ecologists. By choosing some prey over others, predators affect prey population dynamics. According to Optimal Diet Theory (ODT), as prey density increase predators will select more profitable prey. Thereby, prey population dynamics can also affect predator behaviour. Prey profitability involves the prey energetic value, energy costs associated to predation (search, capture and prey handling) and factors that depend on predator behaviour, such as individual prey size, abundance and behaviour. In this study we examined the effect of *Mytilus edulis* association with conspecifics at higher densities on prey selection by *Asterias rubens*. Contrary to ODT prediction, when mussels were tightly clumped in high densities *A. rubens* fed on mussels according to availability. This behaviour resulted in an increase of net profitability caused by the fact that predation destabilizes the association with conspecifics, thereby reducing prey-handling time. We conclude that size selection does not always lead to an improvement of net profit. Size selection is a trade-off between energy yield and predation energy costs, which is affected by prey behaviour. As such, under certain circumstances, increasing prey size does not result in an improvement of net profit for the predator. We discuss the effects of this behaviour on the predator-prey dynamics and the mussel culture industry.

5.1 Introduction

Many predators include a wide array of different prey in their diet. However, they often prefer some species or sizes over others. Prey selection is an important trait which affects not only predators' life story but also mediates the strength of food web interactions, both between predator and prey, and between predators that have a potential diet overlap. Active preference for certain prey items affects prey population structure and community functioning and it is a potential source of density dependency in predator prey interactions, and can hence affect food web stability. For example, by selecting a particular prey, predators can reduce competition with other predators (Menge 1979) or even benefit each other (De Roos *et al.* 2008). Preference for a certain size of individual prey can lead to changes in prey population dynamics. Predators may concentrate their predation effort on juvenile stages decreasing their numbers and recruitment (Van der Veer et al. 1998, van der Heide et al. 2014) or they may aim for a specific sex (Stein 1977), affecting prey population sex ratio.

Optimal diet theory (ODT) is a conceptual framework for understanding why predators choose some prey items over others. According to ODT predators aim to maximize net energy intake per unit time (Macarthur and Pianka 1966, Pyke 1984). In doing so predators aim to prey on the most profitable prey items. Prey profitability or net gain is a complex trait that involves a trade-off between the prey energetic value and the energetic costs involved in predation (search, capture and prey handling, among others) (Brechbühl et al. 2011). Energetic costs depend on predator behaviour, individual prey size and abundance, and also on prey behaviour, size, condition and defence mechanisms or strategies (Dolmer 1998, Sih and Christensen 2001) while the energetic value of prey depends solely on prey size and condition.

According to ODT a predator aims to maximize net gain when preying and therefore selects towards the most profitable prey item. When prey density increases, so does the abundance of optimal prey items and is to be expected that the predator will prey more often or discard other items in favour to the optimal prey items (Pulliam 1974, Charnov 1976, Sih and Christensen 2001). This implies that prey dynamics influences prey selection by predators. However, profit also depends on prey behaviour. Some organisms associate with conspecifics as a defence mechanism against predators, a mechanism that depends on the availability of conspecifics (i.e. abundance) (Duffy and Hay 2001, Bruno et al. 2003, van de Koppel et al. 2008). As with other defence strategies, association with conspecifics may result in changes of predation energetic costs leading to changes in prey profitability (Kishida et al. 2010, Hossie and Murray 2010) which may consequently be reflected in predator prey selection.

The common starfish (*Asterias rubens* L.) is a generalist predator; it preys on a wide array of prey items (Sloan 1980). Starfish, however, have a high preference for blue mussels *(Mytilus edulis* L.) (Gaymer et al. 2001) and are able to control distribution and abundance of mussels in sublittoral habitats (Sloan 1980, Witman et al. 2003). Starfish populations can cause large economic losses to the mussel culture industry (Smaal 2002, Agüera et al. 2012). Starfish prey size is constrained by starfish own size (O'Neill et al. 1983, Gaymer et al. 2004), and they have shown to select the largest available prey within the size range they can target (O'Neill et al. 1983, Hummel et al. 2011).

Mussels form dense matrices where individuals are closely associated (Buschbaum *et al.* 2008). The association of mussels forming clumps or beds can serve as a refuge against predators (Dolmer 1998, van de Koppel et al. 2008, Robles et al. 2009). As mussel density increases so does the complexity of their association with conspecifics because by forming dense matrices they reduce the probability of predators finding an isolated individual (van de Koppel et al. 2008). Knowledge on how these associations affect selective predation is important to determine the predator impact on prey populations (Hughes and Seed 1995). Selective predation by starfish will affect mussel population structure and production and affecting the way this predator impacts cultured and natural mussel resources. However, how mussel density and conspecific association levels may affect starfish mussel size selection is unknown.

This work aims to test the hypothesis that mussel size selection by starfish changes with different levels of prey association while using behavioural observations to explain underlying behavioural mechanisms.

5.2 Material & Methods

5.2.1 Sample collection and storage

Starfish (arm length ranging between 41.3 and 55.2 mm) were collected in the Oosterschelde estuary (the Netherlands) from the subtidal using a small dredge. After 24 hours in a flow-through system, 45 animals were taken and transported to the lab, where they were kept in a 600 l tank and fed with mussels. Mussels (ranging between 5.6 and 33 mm shell length) were collected from the subtidal (collision buoys) at the NIOZ harbour at Texel, The Netherlands. Mussels used in the experiments were kept in a 130 l aquarium.

5.2.2 Experimental design

The experimental set-up consisted of nine trays (50x30x6.5 cm). Trays were arranged in a cascade design in groups of three. All the trays within the same cascade shared a buffer tank (130 l) and biofilter in a closed recirculation arrangement (Figure 5.1). This system was situated in climate-controlled room, kept at a constant temperature of 13ºC and 12/12 hours photoperiod. Each tray was monitored 24 hours from above using a digital video camera; video was recorded for later analysis. Three mussel density treatments (100, 500 and 3000 grams per m^2) were used in each cascade, randomly ordered among the three trays within each cascade. These densities resulted in three levels of association between mussels: loose mussels (100 g m^{-2}), loose + small groups (500 g m^{-2}) and mid to large clumps (3000 g m^{-2}) (Figure 5.2).

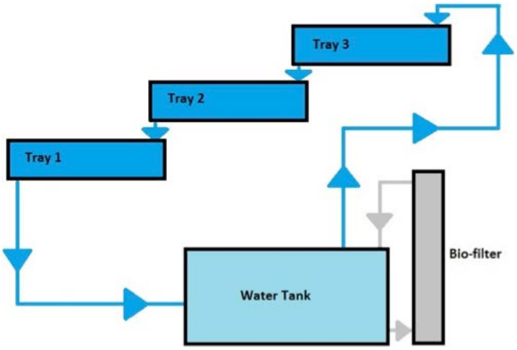


Figure 5.1. Experimental set-up. Arrows show the direction of water circulation.

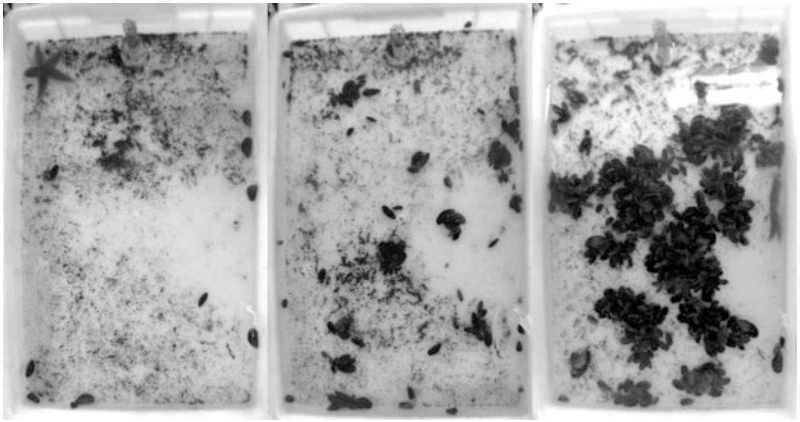


Figure 5.2. Trays with the three different mussel density treatment. From left to right: 100, 500 and 3000 g m^{-2}.

Mussels were taken randomly from the aquaria and manually detached from each other before placing them in the trays. 48 hours prior to the addition of starfish, mussels were placed in the trays for acclimation to allow for self-organization and attachment among themselves or to the substrate. Afterwards, one starfish, of known length and weight was added to each tray. Starfish and mussels were left in the trays over four days and their behaviour recorded by video camera. Each day, eaten mussel shells were removed from the trays and measured to the nearest 0.1 mm using digital callipers. To keep the length distribution and mussel density constant, eaten mussels were replaced by live ones of the same size in the treatments 500 and 100 g m^{-2}. That was not necessary in treatment with 3000 g m^{-2}, in this case we assumed that mussel density was high enough and predation did not cause a significant change in the length distribution of available prey or in the density during four days. After four days starfish were measured again for arm length and weight. This experiment was replicated 3 times with different mussels and starfish. Resulting in observation of predation behaviour of 27 starfish, feeding at three different prey densities (9 individuals per treatment).

A random sample of mussels, of about one hundred individuals, was taken each week from the experiment stock before starting the experiment. These mussels were measured to the nearest 0.1 mm to obtain the length distribution of the mussel used in the experiment. About thirty, selected to cover the whole length distribution, were opened, their flesh content weighed to the nearest 0.001 g and processed for ash free dry weight (AFDW) following the procedure by Kamermans et al. (2009). This data was used to obtain the correlation between mussel shell length, wet weight and AFDW, and was used later on to transform mussel shell length to flesh content and AFDW.

5.2.3 Video Analysis

Video recordings were used to analyse individual starfish behaviour. All video footage available (circa 96 hours per tray/starfish, a total of 2592 hours) was analysed at fast playback using VLC media player v.2.1.3 (www.videolan.org). Time spent searching for prey and handling prey was noted. Prey handling time was defined as the time spent from first contact with the prey until the empty shell was abandoned. Observed attacks (initial starfish-mussel contact to the start of handling the prey) and events of final consumption of captured prey (when the prey was completely consumed and empty shell abandoned) were recorded.

5.2.4 Data Analysis

An ANOVA test confirmed that sea star size was equal among treatments. The difference in the size of consumed mussels between experiment replicates was also tested. Size distribution of consumed mussels in each treatment was compared with the size distribution of available prey using Kolgomorov-Smirnov tests. Barlett’s tests were used to test for homogeneity of variances.

Starfish behaviour at different prey densities was analysed using Fisher’s Least Significance Difference test (LSD) and ANOVA. Behaviour indices analysed included: fraction of total time spent foraging (searching + handling prey), attack rate (number of attacks per hour) and probability of consumption upon attack. In a similar manner, the same tests were used to analyse the effects of the observed behaviour in prey handling time (hour per prey) and profit (mussel AFDW g). All statistical analyses were performed with R 2.15 (www.r-project.org). LSD tests were performed using the function *LSD.test* of the package agricolae (cran.r-project.org/web/packages/agricolae/index.html).

5.3 Results

Starfish size was constant among density treatments (arm length, ANOVA *p* = 0.39). No significant difference in the size of mussels consumed in all treatments among weeks was observed (ANOVA *p* = 0.25). Neither the size of available mussels nor the mussel AFDW content changed between the three replicates of the experiment (ANOVA *p* = 0.22 and *p* = 0.42, respectively). In summary, no time-dependent effect was observed.

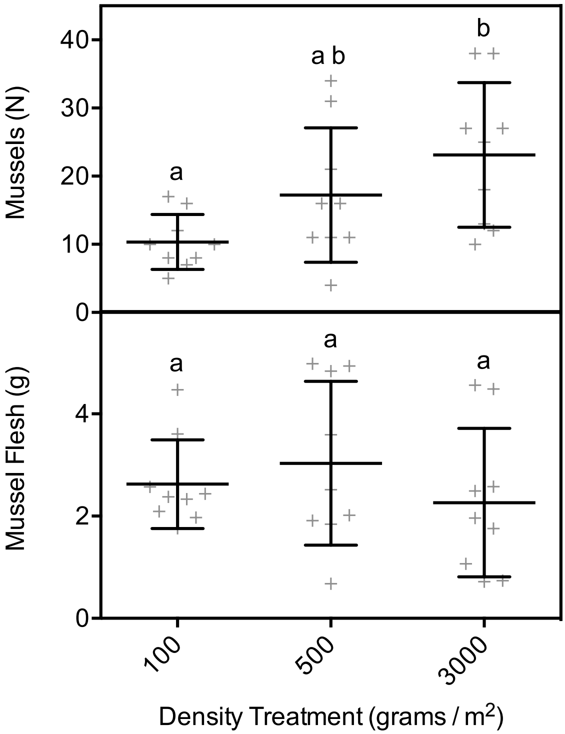


Figure 5.3. Predation rate against mussel density. Rates are for the whole duration of the experiment (4 days). Different letters means significantly different (ANOVA, *p* < 0.05). Bars show mean and SD.

5.3.1 Predation rate and prey selection

Conforming to expectations, decreasing the amount of prey available resulted in a decrease in the number of mussels consumed (Figure 5.3). However, there was no difference between treatments in the total flesh content of consumed mussels (Figure

5.3), because the average size of consumed mussels was larger when density was lower (Figure 5.4); length distribution of consumed mussels at treatments 100 g m^{-2} and 500 g m^{-2} was significantly different from that of treatment 3000 g m^{-2} and the mussels available for consumption in all treatments (Table 5.1).

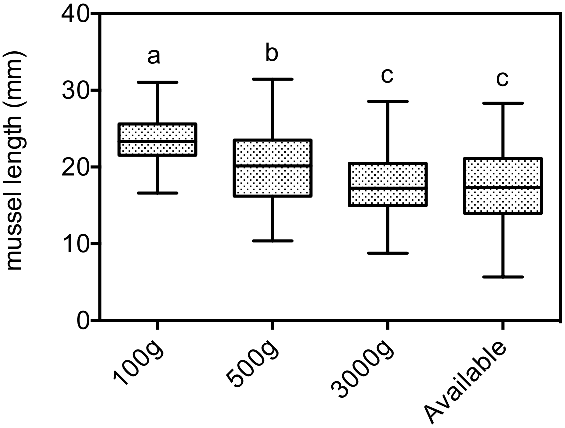


Figure 5.4. Size of consumed in each treatment and available mussel for consumption. Different letters mean significantly different (ANOVA, $p < 0.05$). Boxplot with median and Tukey whiskers.

Table 5.1. Kolmogorov-Smirnov test results comparing the size distribution of consumed mussels in each treatment against the size distribution of mussels available to prey

Compared distributions	Kolmogorov-Smirnov *S*	*p*
Density 100 g m^{-2} vs available	0.5512	<0.001
Density 500 g m^{-2} vs available	0.2315	<0.001
Density 3000 g m^{-2} vs available	0.1544	0.2124
Density 100 g m^{-2} vs Density 500 g m^{-2}	0.3765	<0.001
Density 100 g m^{-2} vs Density 3000 g m^{-2}	0.5934	<0.001
Density 500 g m^{-2}vs Density 3000 g m^{-2}	0.2606	<0.05

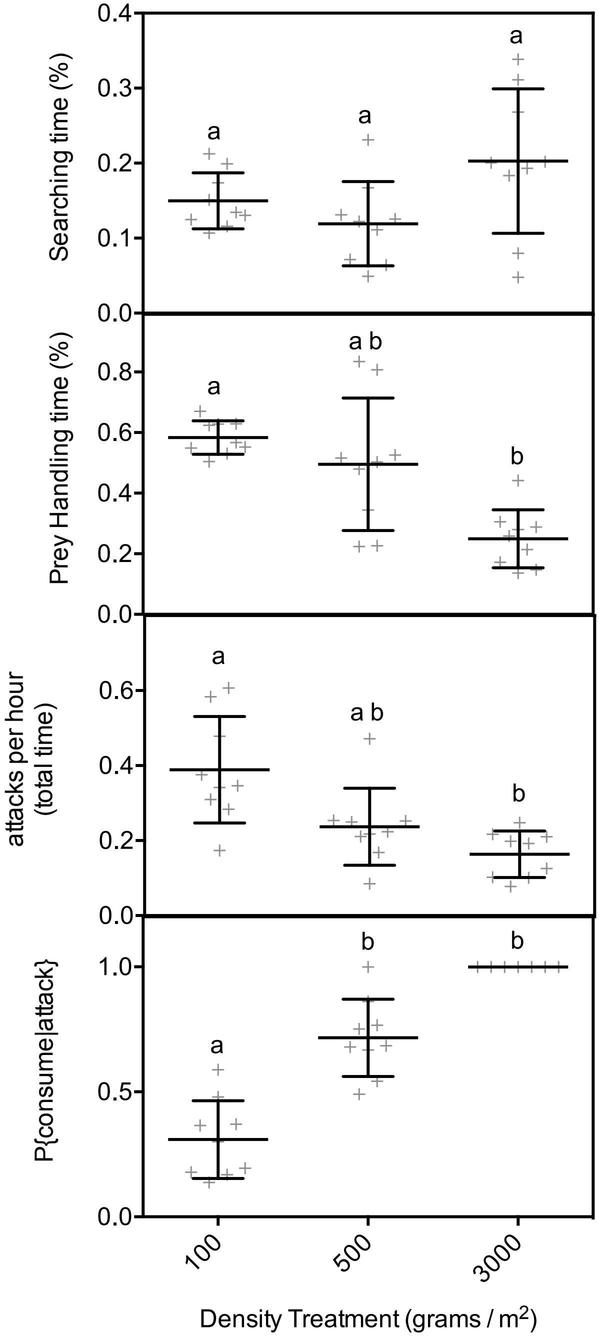


Figure 5.5. Behaviour observations against prey density. Different letters mean significant difference (ANOVA $p < 0.05$). Bars show mean and SD

5.3.2 Prey density, predator behaviour and profit

Video observations showed an increase in foraging activity at lower densities (Figure 5.5), with individuals spending more time handling prey and also covering larger areas. This increase in foraging activity was accompanied by a significant increase in the attack rate (ANOVA $p < 0.01$) (Figure 5.5). However, with the increase in the number of attacks, starfish started to reject prey and the probability of consumption upon attack decreased at lower prey density (Figure 5.5). Contrary to expectations, starfish actively selected larger individuals when densities were lower by rejecting smaller mussels. This behaviour resulted in a change of prey profitability. When density increased, starfish preyed locally removing mussels from the same clump one after another. Handling time per prey decreased (Figure 5.6), as is expected when handling smaller prey. However, our results show that profit was significantly larger at higher densities when no selection was taking place (Figure 5.6).

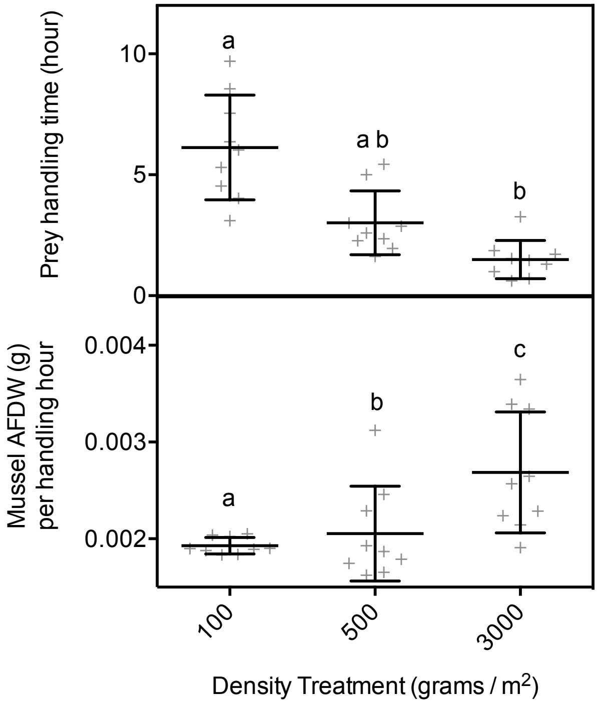


Figure 5.6. Observed effects derived from behavioural changes at different densities. Different letters mean significant difference (ANOVA $p < 0.05$). Bars show mean and SD.

5.4 Discussion

Contrary to what is expected from optimal diet theory (ODT), this study shows that a predator does not necessarily exhibit prey size selection when prey is available at higher densities. Net gain or profit is a complex trait that results from the trade-off between energy intake from prey and the energy costs associated with predation. As such, profit depends on the energy necessary to search and handle prey as well as the prey energetic content. As mussel abundance increases, so does the complexity of its association with conspecifics. This association makes prey harder to dislodge and eat, leading to longer handling time and hence a higher energetic cost of predation. Our results seemingly contradict the expectation, based on ODT, that selection of larger, more profitable prey should occur when prey density is high. However, *A. rubens* optimizes predation by minimizing the amount of energy spent collecting a fixed amount of prey. They do that by preying upon availability, diminishing the handling time/prey but consuming the same amount of flesh that when they select at lower densities. In that manner, the results conform to the underlying principle of ODT, that predators select prey items to optimize net energetic gain.

Prey often exhibits behaviour or adaptations aimed to make predation more difficult (Kishida et al. 2010, Hossie and Murray 2010). In organisms like mussels, association with conspecifics forming clumps is known to have an effect on how predators approach them (Dolmer 1998), with clumping thought to serve as a protection against predation and environmental conditions (van de Koppel et al*.* 2008). This study showed that when starfish preyed on clumps of mussels, they did not select for size. Consequently, they reduced their foraging activity while decimating their prey locally by feeding consistently on the same clump. This change of tactics, compared to that exhibited when preying on low-density mussel trays, resulted in an increase in profit for the starfish (Fig. 6b). The absence of both size selection and increased profit in the high density treatment may be explained by destabilization of the mussel clump caused by the predator itself. Mussels in clumps attach to each other and, as the predator removes individuals, the attachment strength of subsequent prey in that clump is decreased (Aveni-Deforge 2007). Therefore, it may be more efficient, and results in a higher net profit, to attack a smaller mussel in an unstable clump than a larger mussel in a stable clump or strongly attached to substrate. Mussel association leads to profit improvement for the predator, making handling of prey easier when considering subsequent predation in the same spot/clump.

Changing from size-selective to non-selective predation may have important consequences and reflects on mussel population dynamics and mussel bed functioning. Avoiding the selection of larger prey by starfish reduces the probability of larger mussels to be removed. Mussels are thought to attain size refuge under certain circumstances (O'Neill et al. 1983, Agüera et al. 2012), a predator that consistently selects the bigger prey within its predation size-window actively reduces the probability or proportion of prey individuals that will overgrow that maximum size-window and attain size refuge. In contrast, non-selective predation may lead to a higher number of individuals attaining size refuge or reaching maturity, improving the reproduction output (De Roos et al. 2008).

Mussels are cultured for human consumption in many parts of the world, and the effect of prey selection on the prey population dynamics is important for the culture industry. Previous studies suggested that starfish always feed actively selecting bigger prey (O'Neill et al. 1983, Gaymer et al. 2001). By doing so starfish can reduce the mean individual size of the mussel population, and therefore they hamper the ability of the population to grow to commercial valuable size. However, this is not the case at high densities, typically encountered in cultured populations. By feeding in accordance to prey availability, starfish do not affect the individual size of the mussel population, and predation does not affect the growth of the population into commercial size, though it will still cause losses in production.

There are several trade-offs that have an effect in predator behaviour and prey selection. In this study we showed that prey profit changes when mussels are associated with conspecifics, a density dependent trait. This variation in prey profit allows the predator to at least maintain net energetic gain without actively selective predation. A selective process may still happen as starfish may choose prey that are already detached or loosely attached to the clump. We can conclude that size selection does not always lead to an improvement of net profit. On the contrary, under certain circumstances not selecting prey by size results in an improvement of net profit for the predator. Moreover, association with conspecifics may also be a trade-off process and not always results in a defence strategy to make predation more difficult.

References

Agüera, A., M. Trommelen, F. Burrows, J. M. Jansen, T. Schellekens, and A. Smaal. 2012. Winter feeding activity of the common starfish (*Asterias rubens* L.): The role of temperature and shading. Journal of Sea Research 72:106–112.

Aveni-Deforge, K. 2007. Aggregate Behavior of the Blue Mussel, Mytilus Edulis.

Brechbühl, R., J. Casas, and S. Bacher. 2011. Diet choice of a predator in the wild: overabundance of prey and missed opportunities along the prey capture sequence. Ecosphere 2:art133.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution 18:119–125.

Buschbaum, C., S. Dittmann, J.-S. Hong, I.-S. Hwang, M. Strasser, M. Thiel, N. Valdivia, S.-P. Yoon, and K. Reise. 2008. Mytilid mussels: global habitat engineers in coastal sediments. Helgoland Marine Research 63:47–58.

Charnov, E. L. 1976. Optimal foraging: attack strategy of a mantid.

De Roos, A. M., T. Schellekens, T. Van Kooten, and L. Persson. 2008. Stage-specific predator species help each other to persist while competing for a single prey. Proceedings of the National Academy of Sciences of the United States of America 105:13930–13935.

Dolmer, P. 1998. The interactions between bed structure of *Mytilus edulis* L. and the predator *Asterias rubens* L. The Journal of Experimental Marine Biology and Ecology 228:137–150.

Duffy, J. E., and M. E. Hay. 2001. The Ecology and Evolution of Marine Consumer-Prey Interactions. Pages 131–157 *in* M. D. Bertness, S. D. Gaines, and M. E. Hay, editors. Marine Community Ecology.

Gaymer, C. F., C. Dutil, and J. H. Himmelman. 2004. Prey selection and predatory impact of four major sea stars on a soft bottom subtidal community. The Journal of Experimental Marine Biology and Ecology 313:353–374.

Gaymer, C. F., J. H. Himmelman, and L. E. Johnson. 2001. Distribution and feeding ecology of the seastars Leptasterias polaris and Asterias vulgaris in the northern Gulf of St Lawrence, Canada. Journal Of The Marine Biological Association Of The United Kingdom 81:827–843.

Hossie, T. J., and D. L. Murray. 2010. You can’t run but you can hide: refuge use in frog tadpoles elicits density-dependent predation by dragonfly larvae. Oecologia 163:395–404.

Hughes, R. N., and R. Seed. 1995. Criteria for prey size-selection in molluscivorous crabs with contrasting claw morphologies. The Journal of Experimental Marine Biology and Ecology.

Hummel, C., P. Honkoop, and J. van der Meer. 2011. Small is profitable: No support for the optimal foraging theory in sea stars *Asterias rubens* foraging on the blue edible mussel *Mytilus edulis*. Estuarine, Coastal and Shelf Science 94:89–92.

Kamermans, P., M. Blankendaal, and J. Perdon. 2009. Predation of shore crabs (*Carcinus maenas* (L.)) and starfish (*Asterias rubens* L.) on blue mussel (*Mytilus edulis* L.) seed from wild sources and spat collectors. Aquaculture 290:256–262.

Kishida, O., G. C. Trussell, A. Mougi, and K. Nishimura. 2010. Evolutionary ecology of inducible morphological plasticity in predator–prey interaction: toward the practical links with population ecology. Population ecology 52:37–46.

Macarthur, R. H., and E. R. Pianka. 1966. On Optimal Use of a Patchy Environment. The American Naturalist 100:603–609.

Menge, B. A. 1979. Coexistence Between the Seastars *Asterias vulgaris* and *A. forbesi* in a heterogeneous Environment: A non-equilibrium explanation. Oecologia 41:245–272.

O'Neill, S., A. Sutterlin, and D. Aggett. 1983. The effects of size-selective feeding by starfish (*Asterias vulgaris*) on the production of mussels (*Mytilus edulis*) cultured on nets. Aquaculture 35:211–220.

Pulliam, H. R. 1974. On the theory of optimal diets.

Pyke, G. 1984. Optimal Foraging Theory: A Critical Review 15:523–575.

Robles, C. D., R. A. Desharnais, C. Garza, M. J. Donahue, and C. A. Martinez. 2009. Complex equilibria in the maintenance of boundaries: experiments with mussel beds. Ecology 90:985–995.

Sih, A., and B. Christensen. 2001. Optimal diet theory: when does it work, and when and why does it fail? Animal Behaviour 61:379–390.

Sloan, N. A. 1980. Aspects Of The Feeding Biology. Oceanography and Marine Biology: an Annual Review 18:57–124.

Smaal, A. C. 2002. European mussel cultivation along the Atlantic coast : production status , problems and perspectives. Hydrobiologia 484:89–98.

Stein, R. A. 1977. Selective Predation, Optimal Foraging, and the Predator-Prey Interaction Between Fish and Crayfish. Ecology 58:1237.

van de Koppel, J., J. C. Gascoigne, G. Theraulaz, M. Rietkerk, W. M. Mooij, and P. M. J. Herman. 2008. Experimental Evidence for Spatial Self-Organization and Its Emergent Effects in Mussel Bed Ecosystems. Science 322:739–742.

van der Heide, T., E. Tielens, E. M. van der Zee, E. J. Weerman, S. Holthuijsen, B. K. Eriksson, T. Piersma, J. van de Koppel, and H. Olff. 2014. Predation and habitat modification synergistically interact to control bivalve recruitment on intertidal mudflats. Biological Conservation 172:163–169.

van der Veer, H. W., R. J. Feller, A. Weber, and J. I. J. Witte. 1998. Importance of predation by crustaceans upon bivalve spat in the intertidal zone of the Dutch Wadden Sea as revealed by immunological assays of gut contents. The Journal of Experimental Marine Biology and Ecology 231:139–157.

Witman, J. D., S. J. Genovese, J. F. Bruno, J. W. McLaughlin, and B. I. Pavlin. 2003. Massive Prey Recruitment and the Control of Rocky Subtidal Communities on Large Spatial Scales. Ecological Monographs 73:441–462.

Chapter 6

Short-term simulation of starfish population dynamics for impact assessment on mussel populations

Antonio Agüera
Niels Holst
Tim Schellekens
Jeroen M. Jansen
Aad C. Smaal

Abstract

Fisheries management, aquaculture and conservation programs have long been focussed on predation as a key factor controlling the survival of juvenile bivalves, that is when they are more vulnerable. Juvenile blue mussel (*Mytilus edulis*) beds are an important economic and ecological resource subject to predation by the common starfish (*Asterias rubens* L.). *A. rubens* is a keystone predator with a capacity to determine the distribution and abundance of its prey, and it plays an important role controlling the survival of juvenile mussel beds. However, *A. rubens* performance is also limited by environmental factors, such as salinity and temperature, and predation impact is difficult to assess. In this work we developed a model based on laboratory and field observations. The developed model is able to simulate the changes of the *A. rubens* population and its impact on the mussel population, considering current or predicted environmental conditions. We explored model behaviour, verifying its logic and comparing its output with field observations. This model can be used as a tool for fisheries management, aquaculture and also in restoration programs. The model is able to determine the effect of predation by starfish on a short term. This information can be used to assess losses in productivity, survival of natural seedbeds, etc. It can also be used to predict the likely effect of future environmental changes scenarios on the potential impact of *A. rubens* on this important resource.

6.1 Introduction

Bivalve fisheries management, population restoration and aquaculture have long been focused on predation as an important factor controlling juvenile survival. The juvenile stages of bivalves are known to be the most vulnerable phase of their benthic life. Blue mussel (*Mytilus edulis* L.) beds are an important ecological and economical component in many European coastal and estuarine ecosystems. The beds function as a habitat for many other organisms (Buschbaum et al. 2008). Dense aggregations of juveniles (seedbeds) are commercially important, providing seed for bottom culture. Moreover, due to their ecological importance the preservation of seedbeds is the aim of restoration and conservation programs (Agüera et al. 2012). Predation by benthic fauna and birds is thought to play a key role in the survival of both natural and cultured mussel seed aggregations. Among other factors, predation by the common starfish (*Asterias rubens* L.) is known to significantly impact natural seedbeds and seed collectors, playing an important role in the survival of natural seedbeds (Agüera et al. 2012) and hampering seed collector and bottom culture productivity (Smaal 2002)

Asterias rubens L. is considered a keystone predator with the capacity to exert a top down control over its prey population (Witman et al. 2003, Gallagher et al. 2008). It often occurs in high numbers and reacts to massive prey recruitment and other processes that may result in high prey densities (Saier 2001, Witman et al. 2003, Gallagher et al. 2008). Moreover, *A. rubens* exhibits a high predation rate that results in a fast growth rate allowing the population to increase its biomass in a short time (Nichols and Barker 1984, Witman et al. 2003). However, the short-term impact of *A. rubens* does not depend solely on their numbers and biomass. Starfish predation performance can be limited by local environmental conditions such as temperature, salinity and hydrodynamics (Agüera et al. 2012, chapter 3 and 4, this thesis). Local differences and changes in these conditions will affect the impact of *A. rubens* on the mussel seed population. Predicting the impact of a given *A. rubens* population inhabiting a natural seedbed, seed collector, or a culture plot is fundamental for the appropriate management of mussel populations.

Here we present a model that can simulate the biomass dynamics of an *A. rubens* population and its prey demand during autumn-winter, after the new cohort is established. The model can be used as a tool for mussel seed fishery management, conservation programs and optimization of seed collectors harvest. The model links *A. rubens* biomass dynamics to its impact on the mussel population, by determining these dynamics as a function of the predation performance under the local environmental conditions. For this purpose, we applied previous knowledge on the effects of environmental conditions on *A. rubens* and blue mussel performance in a simplified model system. This model was developed from laboratory observations and field data from previous works. Model performance was explored by comparing simulations with real field observations from the Wadden Sea.

6.2 Material & Methods

6.2.1 Model design

The model was designed according to the object-oriented modelling paradigm (Silvert 1993). The ideal object-oriented software is composed of well-defined, loosely-coupled objects, in our case model components, with a minimal interface to other objects (Martin 2006). The model presented here consists of four coupled sub-models simulating the density, both in terms of individuals and biomass, of (1) mussels and (2) starfish, and simulating food acquisition of the starfish (3). A fourth sub-model provides daily readings of seawater temperature and salinity.

The model was formulated as difference equations with a time step of one day. Simulations were carried out using Euler integration, with an integration step of 1 day. Functional relationships and parameter values were estimated from published literature and model predictions were compared against independent field data. The ecological variables and parameters of the model are shown in Table 1, whereas regression coefficients describing functional relationships are given in text. Biomass is in wet weight throughout.

Table 6.1. Model variables.

State variables	**Symbols**	**Units**
Density of biomass	M_m, M_s	g m^{-2}
Density of individuals	N_m, N_s	n m^{-2}
Individual weight	W_m, W_s	g n^{-1}
Finite rates		
Growth	M_m^g, M_s^g	g m^{-2} day^{-1}
Loss by competition	N_m^c, M_m^c	n m^{-2} day^{-1}, g m^{-2} day^{-1}
Loss by predation	N_m^p, M_m^p	n m^{-2} day^{-1}, g m^{-2} day^{-1}
Predation demand	D_s	g prey m^{-2} day^{-1}
Relative rates		
Base respiration	z_s	g g^{-1} day^{-1}
Predation demand	d_s	g prey g $predator^{-1}$ day^{-1}
Search efficiency	s	unitless
Energy budget proportions		
Egestion	β	unitless $\in[0;1]$
Conversion cost	λ	unitless $\in[0;1]$
Scaling factors		
Temperature	τ_{m}, τ_{s}	unitless $\in[0;1]$
Salinity	σ_{m}, σ_{s}	unitless $\in[0;1]$
Tidal flow (hydrodynamics)	υ_{s}	unitless $\in[0;1]$
Constants		
Initial density of biomass	$M_m[0]$, $M_s[0]$	g m^{-2}
Initial density of individuals	$N_m[0]$, $N_s[0]$	n m^{-2}
Carrying capacity	K_m	g n^{-1}
Driving variables		
Temperature	T	°C
Salinity	S	PSU
Tidal flow maximum velocity	V	cm s^{-1}

Notes: g are all g wet weight

The model was implemented in an open-source C++ program using the Universal Simulator framework version 1.50 (Holst, 2013). Model source code, an installation file to run the model on Microsoft Windows and a manual can be found on www.ecolmod.org and in the electronic appendix to this paper.

6.2.2 Boundary conditions

For this model the mussel bed ecosystem is simplified as a system with one predator (starfish, *A. rubens*) that preys exclusively on one prey (mussels, *M. edulis*). The model simulates the population dynamics during autumn-winter in a mussel seedbed in the Wadden Sea. For further simplification, each population was assumed to consist of one uniform cohort, all individuals having the same body mass. This assumption is backed by the observation that *A. rubens* blooms are generally composed of only one cohort (Guillou 1996; Uthicke, Schaffelke & Byrne 2009), and that seedbeds are by definition only one cohort (Agüera et al. 2012).

6.2.3 Mussel model

Mussel abundance (N_m, n m^{-2}), initially set by the seeding or spat fall event, may decrease over time due to losses caused by intraspecific competition (thinning) (N_m^c n m^{-2} day^{-1}) and starfish predation (N_m^p, n m^{-2} day^{-1}). We assumed that mussel abundance cannot increase outside the reproductive season, because mussels are sessile. Mussel biomass (M_m, g m^{-2}) can increase due to somatic growth (M_s^g, g m^{-2} day^{-1}), and decreases because of intraspecific competition (M_m^c, g m^{-2} day^{-1}) and predation (M_m^p, g m^{-2} day^{-1}). That results in mussel density and abundance changing per day as follows:

$$\Delta N_m = -N_m^c - N_m^p \tag{1}$$

$$\Delta M_m = M_m^g - M_m^c - M_m^p \tag{2}$$

6.2.3.1 Mussel growth

The maximum growth rate of mussels ($g_m^{\max}$, g g^{-1} day^{-1}) decreases with increasing mussel size (W_m, g) (Figure 6.1),

$$g_m^{\max} = aW_m^b, \tag{3}$$

where $W_m = \frac{M_m}{N_m}$, a = 0.0159±0.0048 and b = -0.486±0.409.

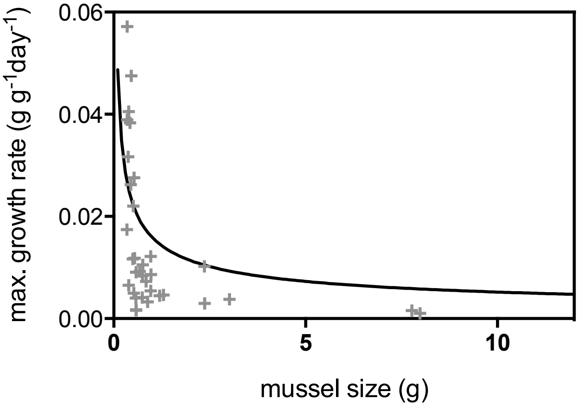


Figure 6.1. Points represent growth dates yielded from project PRODUS field observations (Glorius *et al.* 2013). Curve represents formula (3) with the given parameters.

This rate was calculated from field data from the Wadden Sea obtained during the project PRODUS (Smaal *et al.* 2013) (Figure 6.1), and the regression represent the quantile regression leaving 90% of the observations below the line, in the attempt to assess the limiting effect of size in the observed growth rate. These maximum observed rates were assumed to occur under optimal conditions of temperature and salinity. Temperatures below the optimal 18 °C reduce this maximum growth rate, which we expressed by a scaling factor between 0 and 1 (Figure 6.2),

$$\tau_m = \frac{e^{aT-b}}{1+e^{aT-b}}, \tag{4}$$

where a = 0.40831±0.99 and b = -4.1573±1.234

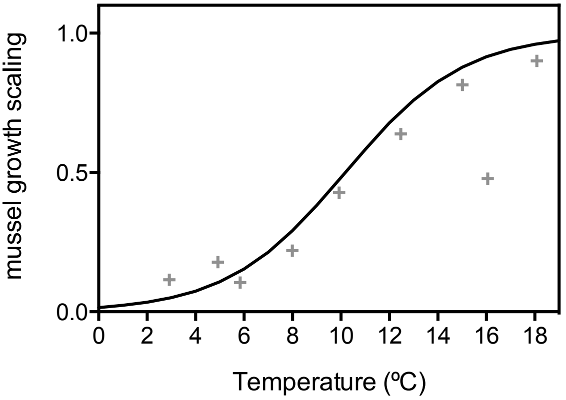


Figure 6.2. Temperature scaling factor for mussel growth. Points yielded from laboratory data by (Almada-Villela et al. 1982). Curve represents formula (4) with the given parameters. Parameters were obtained by quantile regression leaving 90% of the observations below the line.

Similarly we applied a scaling factor to account for sub-optimal salinity, below 32 PSU (Figure 6.3),

$$\sigma_m = \frac{e^{aS-b}}{1+e^{aS-b}}, \qquad (5)$$

where a = 0.4976±0.404 and b = -11.798±2.7164.

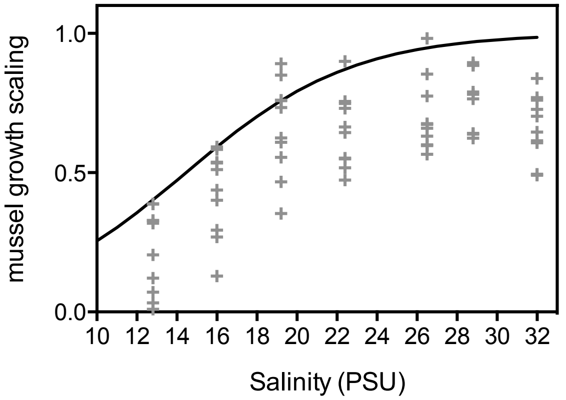


Figure 6.3. Salinity scaling factor for mussel growth. Points yielded from laboratory data by (Almada-Villela 1984). Curve represents formula (5) with the given parameters. Parameters were obtained by quantile regression leaving 90% of the observations below the line

Population growth is bounded by the carrying capacity, K_m = 15.000 g/m^2, which was the maximum observed density in the Wadden Sea during autumn between years

2005-2007 (Ens *et al.* 2007). The finite rate of growth (M_m^g, g m^{-2} day^{-1}) was calculated using carrying capacity as in a logistic growth model, and taking the limiting factors above into account,

$$M_m^g = g_m^{\max} \tau_m \sigma_m \left(1 - \frac{M_m}{K_m}\right) \tag{6}$$

6.2.3.2 Mussel competition

The maximum possible number of mussels per area ($N_m^{\max}$, n m^{-2}) depends on mussel size (Figure 6.4),

$$N_m^{\max} = a W_m^b \tag{7}$$

where a = 3330±150.13 and b = -0.871±0.0872.

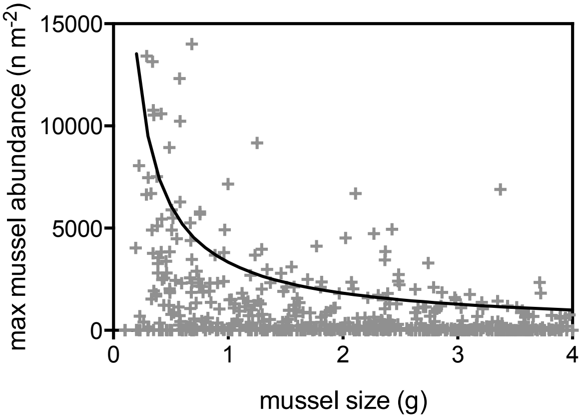


Figure 6.4. Points represent observed abundance and size in the Wadden Sea during Autumn (Ens *et al.* 2007). Curve represents formula (7) with the given parameters. Parameters were obtained by quantile regression leaving 90% of the observations below the line.

This function was obtained by regression on field observations in the Wadden Sea during autumn (Ens *et al.* 2007)

This means that, once we have taken the loss to predation (N_m^p) into account, the remaining mussels cannot exceed this limit on density. From this follows the loss of mussel individuals caused by intraspecific competition (self-thinning),

$$N_m^c = \left\lfloor N_m - N_m^p - N_m^{\max} \right\rfloor_0 , \tag{8}$$

where the notation $\lfloor ... \rfloor_c$ enforces a minimum value of *c*. Thus, by way of individual mussel mass (W_m), is translated into the accompanying loss of mussel biomass,

$$M_m^c = W_m N_m^c \tag{9}$$

6.2.4 Starfish model

Starfish abundance (N_s, n m^{-2}) was assumed constant in the model, whereas starfish mass may increase by the intake of mussel prey (M_s^p, g m^{-2} day^{-1}) and decrease by respiration (M_s^r, g m^{-2} day^{-1}). These assumptions results in starfish density and abundance changing per day as follows

$$\Delta N_s = 0 \tag{10}$$

$$\Delta M_s = M_s^g - M_s^r \tag{11}$$

6.2.4.1 Starfish functional response

The maximum growth rate of starfish ($g_s^{\max}$,g g^{-1} day^{-1}) decreases with starfish body mass (W_s, g), under optimal conditions (laboratory determination with ample food at 15ºC, 30 PSU) (Figure 6.5),

$$g_s^{\max} = aW_s^b \tag{12}$$

where *a* = 0.181±0.029 and *b* = -0.516±0.067

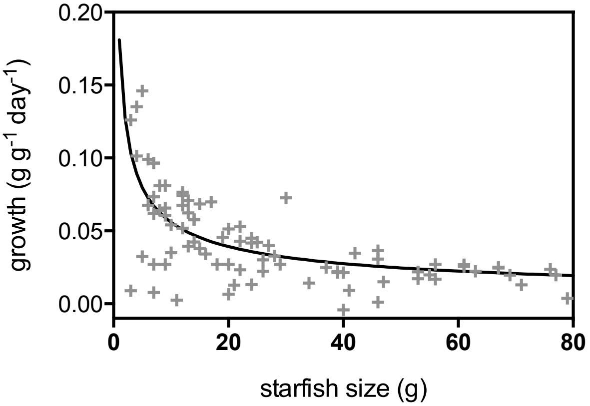


Figure 6.5. Starfish growth rate at size. Points are laboratory observations under optimal condition, (Agüera et al. *Unpublished data*). Curve represents formula (12) with the given parameters. Parameters were obtained by non-linear least squares regression.

The basal metabolism of starfish (z_s, g g^{-1} day^{-1}) increases with temperature (Figure 6.6),

$$z_s = ae^{bT} \tag{13}$$

where a = 0.00161±0.0003 and b = 0.103±0.021

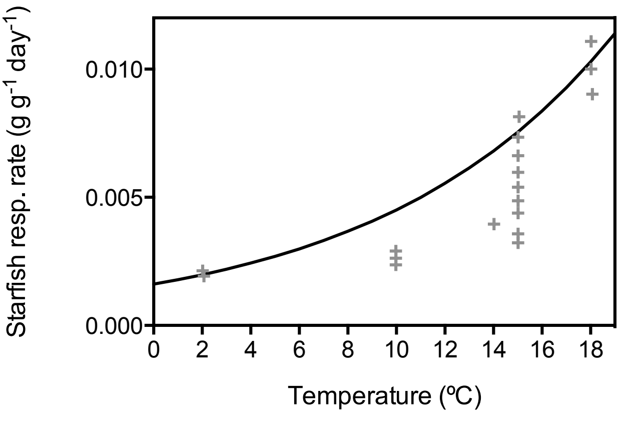


Figure 6.6. Starfish respiration rate at temperature. Data are laboratory observations of starved individuals (Fonds et al. 1989). Curve represents formula (13) with the given parameters. Parameters were obtained by quantile regression leaving 90% of the observations below the line.

We assumed that all mussel biomass, except the shell, is used to build starfish biomass at a conversion cost of λ = 0.842 g starfish biomass built per consumed g of mussel biomass (Figure 6.7).

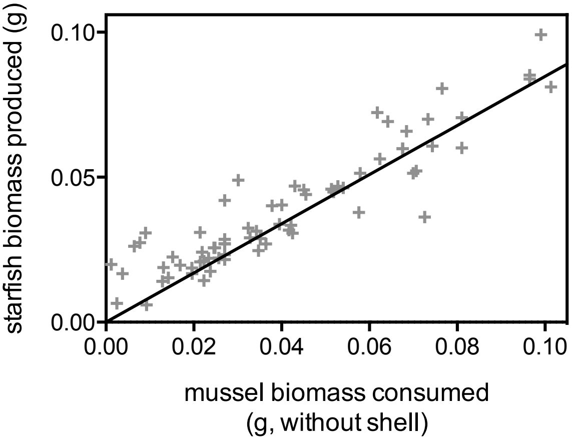


Figure 6.7. Conversion costs from consumed mussel flesh to starfish biomass. Points are laboratory observations under optimal condition, (Agüera et al. *Unpublished data*). Line is a linear regression with zero intercept and slope = 0.842±0.0213.

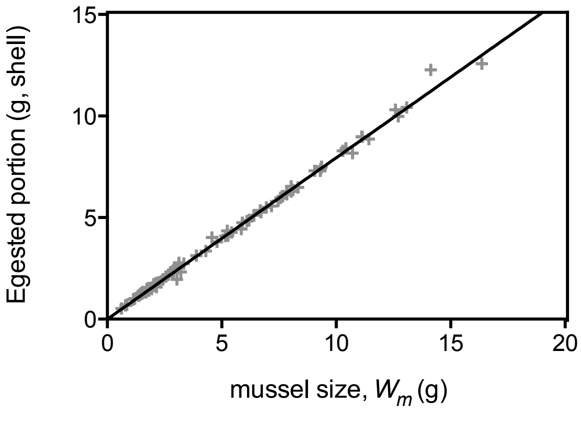


Figure 6.8. Mussel weight egested by starfish during predation (shell) at mussel size W_m. Line represent s linear regression with zero intercept and slope = β.

The food demand calculated at this point is in the form of mussel flesh (g). However, live mussels and field data on mussel density is given as total biomass including shell weight that is egested (i.e. not consumed) by starfish when feeding on mussels. To calculate the real demand of mussel biomass for the starfish population is necessary to escalate the flesh demand to total mussel weight. To do so the shell proportion was set to β=0.853±0.0031 of total mussel weight. This value was estimated from samples from the Wadden Sea (figure 8).

The total demand rate for predation of the starfish population (d_s, g mussel killed / g starfish day) thus becomes

$$d_s = \frac{g_s^{\max} + z_s}{\lambda(1-\beta)} \tag{14}$$

As long as this demand is fulfilled by prey supply, the predator will attain its maximum growth rate; otherwise the growth rate will be less. The finite demand over one day,

$$D_s = d_s M_s \tag{15}$$

is used to calculate the actual supply (S_s, g m^{-2} day^{-1}) by way of the Gutierrez-Baumgärtner functional response (Gutierrez 1992),

$$S_s = D_s \left\{ 1 - \exp\left(\frac{-sM_m}{D_s} \right) \right\} \tag{16}$$

where s is the starfish search rate. According to this functional response, as prey gets limiting, the supply gets proportional to prey density, $S_s \to sM_m$. With unlimited prey, the supply approaches the demand, $S_s \to D_s$.

Search rate account for the capacity of the predator to find and capture prey. For starfish under optimal conditions this value is circa one. However, environmental conditions such as temperature, salinity and hydrodynamics are known to limit starfish capacity to move and prey (Agüera *et al.* 2012, chapter 3 and 4). This decrease in the predation performance was modelled by reducing *s*. We expressed the effect of suboptimal temperature, salinity and hydrodynamic conditions on *s* as scaling factors between 0 and 1, in the same fashion that mussel growth was scaled.

The effect of sub-optimal temperature on *s* is scaled by (Figure 6.9)

$$\tau_m = \frac{e^{aT-b}}{1+e^{aT-b}}, \tag{17}$$

where a = 0.5278±0.724 and b = -3.9662±0.675.

For sub-optimal salinity the scaling factor is given by (figure 6.10)

$$\sigma_m = \frac{e^{aS-b}}{1+e^{aS-b}}, \tag{18}$$

where a = 0.743±0.24 and b = -16.831±5.37.

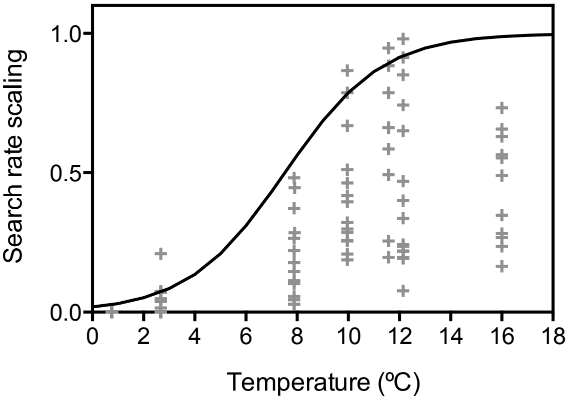


Figure 6.9. Temperature scaling factor for *s*. Points obtained from activity observations in mesocosm experiment (Agüera *et al.* 2012). Curve represents formula (17) with the given parameters. Parameters were obtained by quantile regression leaving 90% of the observations below the line.

For the effect of flow velocities due to tidal movement, we calculated a scaling factor considering one tidal displacement (12 hours), This value was only calculated once and is locality specific, depending solely of the maximum tidal flow velocity registered for the location as such the scaling factor was calculated as follows:

$$\upsilon_S = \int_{t=0}^{t=12} \frac{e^{a+bV\sin(2\pi/12)}}{1+e^{a+bV\sin(2\pi/12)}}, \tag{19}$$

where V is the daily maximum velocity of tidal movement (cm s^{-1}), a = 3.4436±0.287, b = -0.1694±0.012 and t is time in hours (Figure 6.11). The sigmoidal expression obtains the activity level at flow velocity, however flow velocity changes with the tide in cycles of 12 hours. The integral aims to calculate a scaling factor for searching, considering that flow velocity changes with the tide. To do so we assumed that tide is a sinusoidal wave and calculate searching rate considering the activity level at each flow velocity during the tidal movements from its minimum to the maximum, which is given by *V*.

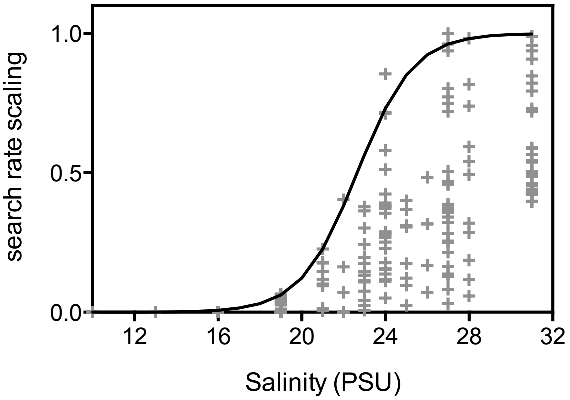


Figure 6.10. Salinity scaling factor for *s*. Data points from activity observation in mesocosm experiment (chapter 3, this thesis). Curve represents formula (18) with the given parameters. Parameters were obtained by quantile regression leaving 90% of the observations below the line.

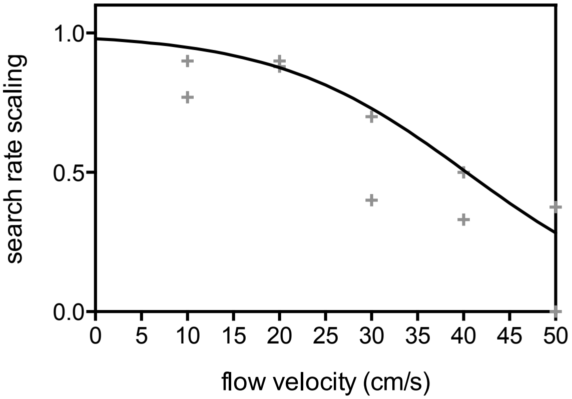


Figure 6.11. Tidal flow velocity scaling factor for *s*. Data points are yielded from observations in flow tank (chapter 4, this thesis). Curve represents sigmoidal equation integrated in formula (19) with the given parameters. Parameters were obtained by quantile regression leaving 90% of the observations below the line.

Data used to assess this scaling was based on a mussel density of 1000 g m^{-2}. However, the effects of hydrodynamics on starfish activity are in part determined by the capacity of mussels to reduce hydrodynamic stress (Folkard and Gascoigne 2009). To consider the effect of mussel density in amelioration of flow, this model uses a function to increase or decrease u_s considering current mussel biomass (M_m),

$$\upsilon_s^m = \upsilon_s + \left[\left(\frac{M_m - 1000}{5000 - 1000}\right)(1 - \upsilon_s)\right] \tag{20}$$

The model considers that flow have hardly any effect on starfish when $M_m > 5000$ g m^{-2}.

Search rate under applying environmental conditions is then given as

$$s = \tau_s \sigma_s \upsilon_s^m \tag{21}$$

The supply (eq. 16) is allocated to growth (M_s^g, g m^{-2} day^{-1}) after subtracting the egested part ($ß$) and respiration (M_s^r, g m^{-2} day^{-1}),

$$M_s^g = (1 - \beta)S_s - M_s^r \tag{22}$$

$$M_s^r = z_s M_s \tag{23}$$

If no prey is available this model assumes that starfish starve at a rate of z_s. Starfish are well-known to be able to withstand prolonged starvation (Jangoux and Impe 1977).

6.2.4 Model exploration

We explored the model performance by comparing simulations with field observations. Considering local environmental conditions.

6.2.4.1 Field observations

Between the years 2006 and 2012 a total of 40 plots in the Wadden Sea measuring 200 by 200 m were closed to the mussel fishery as part of the project PRODUS[1] (Sustainable Shellfish Culture) (Glorius et al 2013, Smaal et al 2013). This project monitored the mussel population and associated fauna, including *A. rubens*. To do so, each plot was sampled at least twice a year, just before the mussel seed fishing season in autumn and again in spring. Each plot sample consisted of two suction dredges covering about 30 m^2 within each plot. A small net mesh (5mm) allowed the dredge to capture even the smaller starfish and mussel individuals. Biomass (g m^{-2})

---

[1] http://www.wageningenur.nl/en/Expertise-Services/Research-Institutes/imares/Projects/PRODUS-Sustainable-shellfish-culture.htm

and abundance (n m^{-2}) of mussels and *A. rubens* were obtained from these dredges. Plots where settlement and formation of new mussel beds were observed in autumn sample were sampled again during the same autumn. As such, several plots were sampled twice between September and November 2009 and between September and December 2010, in this study we used those consecutive autumn samples to explore the model. A total of 21 sample pairs (t_0 = first autumn sample + t_1 = consecutive autumn samples) were obtained. These consecutive samples allowed us short term monitoring of mussel beds in the field. Time between t_0 and t_1 samples of the same plot varied between 60 and 100 days. Conditions recorded at t_0 (location, sampling date, M_s, N_s, M_m and N_m) were used as initial values. Simulations lasting between t_0 and t_1 were performed for each of the 21 samples.

6.2.4.2 Environmental data

The present model requires the input of environmental data in the form of daily mean temperature and salinity. This data was provided by a numerical model that describes freshwater circulation within the Wadden Sea (Duran-Matute et al. 2014). Environmental data covered the years 2009 to 2011 and consisted of daily mean salinity and temperature at the bottom for the locations of the 40 PRODUS plots.

6.2.4.2 Model predictions of mussel and starfish growth

Conditions recorded in the field at t_0 (location, sampling date, mussel and starfish density and abundance) were used as simulations initial values. Simulations lasting the time between t_0 and t_1 were performed for each one of the 21 samples. Values of mussel and starfish density and abundance obtained at the end of the simulations were compared with the sample taken in the field at t_1. Coefficients of determinations (r-squared) and residual sum of squares (RSS) were assessed to evaluate the capacity of the model to describe field situations.

6.3 Results

The model simulates daily changes in mussels and starfish density and abundance. These simulations can be used to assess how a mussel bed, defined by its density and abundance, changes under the predation of a population of *A. rubens*, defined also by its density and abundance. The model output consists of several variables (Table 6.1): density, abundance, mean individual size (calculated as the density/abundance) and growth rate for both mussels and *A. rubens*. Additionally, it also contains intermediary variables as mussel mortality associated to *A. rubens* predation and mussel mortality due to thinning.

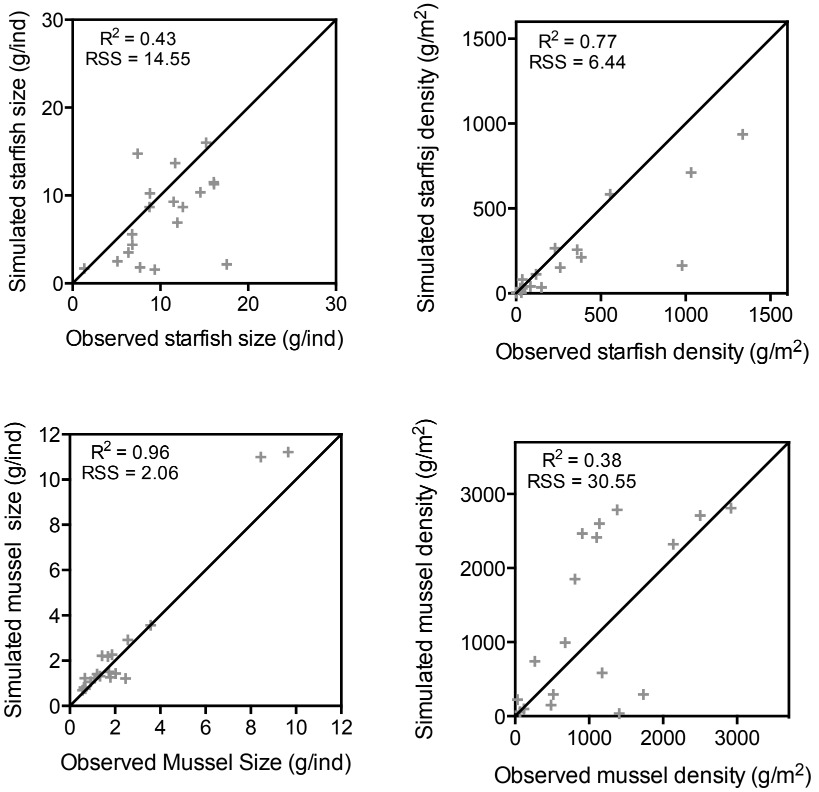


Figure 6.12. Predicted size and biomass of starfish and mussels plotted against observed values in the field. Line represent x=y

In the simulations *A. rubens* yields a final size that describes most of the variation observed in the field (Figure 6.12). Results improve when comparing simulated results with observations of *A. rubens* population biomass (Figure 6.12). Observed mussel density (g m^{-2}) was also well predicted by model simulations (Figure 6.12).

6.4 Discussion

Predation by starfish is an important factor determining survival of mussel seedbeds during their first autumn and winter. A tool that is able to predict the impact of *A. rubens* population on juvenile mussel beds is necessary to improve the management of this important ecological and economic resource. Control is a key factor and having prior knowledge on the impact of a known starfish population inhabiting a mussel seedbed, a seed collector or a culture plot allows optimizing the measures against starfish, saving costs and reducing production losses. The model presented here describes the dynamics of *A. rubens* and its predation pressure on the mussel population. The predation rate is determined by predator biomass and the limitations imposed by the environmental conditions. Despite the complexity of the Wadden Sea environment, this simulation model is able to describe a great deal of the observed variability of mussel and starfish population changes during autumn by simulating predation by starfish and growth of both starfish and mussels. Model analysis showed that the model is able to simulate *A. rubens* biomass dynamics, based only food intake. This is a robust proxy to predation pressure, as it approximates the amount of mussels that are removed by *A. rubens*, i.e. assessing its impact on the mussel population. *A. rubens* intake is heavily influenced by the environmental conditions considered by the model and by the abundance of the mussels. The model describes the changes of the mussel population under the same environmental conditions with the same level of accuracy as that of *A. rubens*.

Model analysis showed that *A. rubens* predation and mussel self-thinning account for a large part of the variation in mussel biomass observed in the field (Figure 6.12). Mussel mortality is determined by other factors not considered in this model, such as predation by shore crabs (Kamermans et al. 2009) and diving ducks (Leopold et al. 2001). Mussel food was assumed unlimited but may be limiting in nature (Smaal and van Stralen 1990). Part of the discrepancy between model predictions and observations may be caused by the omission of these model components, but may also result from sampling error due to the patchy distribution of mussels and starfish within the mussel bed. More field data on mussel and starfish growth and environmental conditions could be used to further improve and validate this model that is mostly based on laboratory observations. Further calibration and validation with more field data should produce more accurate parameters and predictions. Model simulations rely on environmental conditions that are of course variable and may not be predictable. Use of historical environmental data may help to set different scenarios to evaluate the changes in mussel and starfish population under more or less favourable conditions.

Knowledge of changes of mussel biomass changes due to *A. rubens* predation is useful for mussel bed management. Within its limitations this model may allow fisheries and culture management to make decisions on harvesting of wild and cultured seed in order to minimize losses. It can, for example, be used to predict how long it takes for starfish to reduce mussel biomass to a certain level under different environmental conditions. Mussel farmers may therefore use the model outcome to indicate whether it is necessary to take measures to reduce starfish biomass from a culture plot or not, minimizing losses in mussel productivity. The model may also help to test future or alternative scenarios of environmental change, such as changes in precipitation regimes, allowing us to foresee the effects of those environmental changes on the role of *A. rubens* impact on mussel seedbed stability.

References

Agüera, A., M. Trommelen, F. Burrows, J. M. Jansen, T. Schellekens, and A. Smaal. 2012. Winter feeding activity of the common starfish (*Asterias rubens* L.): The role of temperature and shading. Journal of Sea Research 72:106–112.

Almada-Villela, P. C. 1984. The effects of reduced salinity on the shell growth of small *Mytilus edulis*. Journal Of The Marine Biological Association Of The United Kingdom 64:171–182.

Almada-Villela, P. C., J. Davenport, and L. D. Gruffydd. 1982. The effects of temperature on the shell growth of young mytilus edulis L. The Journal of Experimental Marine Biology and Ecology 59:275–288.

Buschbaum, C., S. Dittmann, J.-S. Hong, I.-S. Hwang, M. Strasser, M. Thiel, N. Valdivia, S.-P. Yoon, and K. Reise. 2008. Mytilid mussels: global habitat engineers in coastal sediments. Helgoland Marine Research 63:47–58.

Duran-Matute, M., T. Gerkema, G. J. de Boer, J. J. Nauw, and U. Gräwe. 2014. Residual circulation and fresh-water transport in the Dutch Wadden Sea: a numerical modelling study. Ocean Science Discussions 11:197–257.

Ens, B. J., J. Craeymeersch, F. Fey-Hofstede, H. J. L. Heessen, A. C. Smaal, A. G. Brinkman, R. Dekker, J. Van Der Meer, and M. V. Stralen. 2007. Sublitorale natuurwaarden in de Waddenzee. Wageningen IMARES. C077/07

Folkard, A. M., and J. C. Gascoigne. 2009. Hydrodynamics of discontinuous mussel beds: Laboratory flume simulations. Journal of Sea Research 62:250–257.

Fonds, M., M. Hup, and P. van der Puyl. 1989. Metabolism, food consumption, growth and food conversion efficiency in the sea star *Asterias rubens* L. NIOZ unpublished data.

Gallagher, T., C. A. Richardson, R. Seed, and T. Jones. 2008. The Seasonal movement and abundance of the starfish *Asterias rubens* in relation to mussel farming practice: a case study from the Menai Strait, UK. Journal of Shellfish Research 27:1209–1215.

Glorius, S., A. Rippen, M. R. van Stralen, and J. M. Jansen. 2013. Effecten van mosselzaadvisserij op het bodemleven van de Waddenzee. IMARES Wageningen UR. Report C162/12

Gutierrez, A. P. 1992. Physiological Basis of Ratio-Dependent Predator-Prey Theory: The Metabolic Pool Model as a Paradigm. Ecology 73:1552.

Jangoux, M., and E. V. Impe. 1977. The annual pyloric cycle of *Asterias rubens* L. (ECHINODERMATA: ASTEROIDEA). The Journal of Experimental Marine Biology and Ecology 30:165–184.

Kamermans, P., M. Blankendaal, and J. Perdon. 2009. Predation of shore crabs (*Carcinus maenas* (L.)) and starfish (*Asterias rubens* L.) on blue mussel (*Mytilus edulis* L.) seed from wild sources and spat collectors. Aquaculture 290:256–262.

Leopold, M. F., R. K. H. Kats, and B. J. Ens. 2001. Diet (preferences) of Common Eiders *Somateria mollissima*. Wadden Sea Newsletter:25–31.

Martin, R. C. 2006. Agile software development: principles, patterns, and practices. Prentice-Hall, Upper Saddle River, NJ.

Nichols, D., and M. F. Barker. 1984. Growth of juvenile *Asterias rubens* L.(ECHINODERMATA:ASTEROIDEA) on an intertidal reef in southwestern Britain. The Journal of Experimental Marine Biology and Ecology 78:157–165.

Saier, B. 2001. Direct and indirect effects of seastars *Asterias rubens* on mussel beds (*Mytilus edulis*) in the Wadden Sea. Journal of Sea Research 46:29–42.

Silvert, W. 1993. Object-oriented ecosystem modelling. Ecological Modelling 68:98–118.

Smaal, A. C. 2002. European mussel cultivation along the Atlantic coast : production status , problems and perspectives. Hydrobiologia 484:89–98.

Smaal, A. C., and M. R. van Stralen. 1990. Average annual growth and condition of mussels as a function of food source. Pages 179–188 *in* D. McLusky, V. N. de Jonge, and J. Pomfret, editors. North Sea - Estuaries Interactions. Springer Netherlands, Dordrecht.

Smaal A.C., J. Craeymeersch, J. Drent, J.M. Jansen, S. Glorius & M.R. van Stralen, 2013. Effecten van mosselzaadvisserij op sublitorale natuurwaarden in de westelijke Waddenzee: samenvattend eindrapport. Rapport C006/13 PR1

Witman, J. D., S. J. Genovese, J. F. Bruno, J. W. McLaughlin, and B. I. Pavlin. 2003. Massive Prey Recruitment and the Control of Rocky Subtidal Communities on Large Spatial Scales. Ecological Monographs 73:441–462.

Chapter 7

General Discussion

The aim of this thesis was to assess the role *Asterias rubens* in mussel bed stability and to determine which factors are affecting it. The practice background is the dual ecological and economic value of young mussel beds (seedbeds) in the Wadden Sea, which require an assessment of their stability. As such, this study aims to contribute to an improvement management of the seed fishery and of restoration programmes. A major question regards the determination of stable and unstable mussel seedbeds (Chapter 1). Currently seedbeds considered unstable are fished before winter because they are supposed to disappear while stable seedbeds are not fished in autumn. The problem remains in what decision to take when *A. rubens* presence is recorded in seedbeds falling within the intermediate stability categories (Figure 1.2). In those cases it is necessary to determine what is the role of *A. rubens* in the mussel bed stability, so that the decision of fishing or not fishing can be taken on a more profound basis. Therefore understanding factors that determine the interaction between predator and prey is crucial for better management.

Asterias rubens is an important shellfish predator, with the capacity to exert top down control its prey population (Menge 1982). This characteristic has made of *A. rubens* a recognized pest for the shellfish culture industry (Hancock 1955, Barbeau et al. 1996, Agüera et al. 2012). Among its various prey *A. rubens* exhibits a preference for the blue mussel (Hancock 1955). *A. rubens* is often associated with mussel culture activities (Gallagher et al. 2008) and wild mussel beds (Witman et al. 2003). *A. rubens* can be present at very high densities on mussel beds and has a great capacity to decimate its prey (Chapter 1 and Chapter 2) resulting in a fast decrease of the prey population (Witman et al. 2003). Such predation pressure results in large losses in productivity for the mussel culture industry and a decrease of abundance in wild populations (Smaal 2002, Smaal et al. 2014).

The impact of *A. rubens* on its prey population is defined by the capacity of the present population to remove prey. This capacity depends upon predator individual predation rate and also the density of its population. Distribution and abundance are both constrained by environmental conditions, such as salinity (Chapter 3) and hydrodynamics (Chapter 4). Individual predation rate is further limited by osmotic stress caused by variable salinity (Chapter 3), water temperature (Chapter 2) and flow velocity (Chapter 4). Predation rate is also affected by prey availability, prey size and prey behaviour (Chapter 5).

In this thesis research was conducted with the aim of bridging knowledge gaps limiting our understanding of how environmental salinity, temperature and hydrodynamics affect the capacity of *A. rubens* to remove prey. Giving answers to the four questions regarding the performance of *A. rubens*

1. How important is *A. rubens* predation during winter? What is the role of temperature?
2. How is osmotic stress affecting *A. rubens* feeding rate?
3. How does *A. rubens* perform under high velocity flows? Does it affect its feeding rate?
4. How does mussel association with conspecifics affect *A. rubens* feeding rate?

Research focused on the quantification of predation rate under seasonal temperature change (Chapter 2) and under different levels of osmotic and hydrodynamic stress (Chapter 3 and 4). We further researched on the interaction of *A. rubens* with the mussel and the effect of prey behaviour on *A. rubens* feeding strategy (Chapter 5). In the end the results obtained were integrated with existing literature to create a simulation model able to assess the changes of mussel biomass under predation by *A. rubens* considering environmental conditions and the interaction with the mussel population. This model, research results and existing literature can now be used to analyse the Wadden Sea situation and provide answers to the question behind this thesis: **What is the role of *A. rubens* in mussel seedbed stability?**

7.1 Distribution and seasonal abundance of *Asterias rubens* in the Wadden Sea

In the Wadden Sea, *Asterias rubens* is mainly distributed in the subtidal. Its distribution, as it often occurs in other locations, is closely related to the presence of mussels. However, abundance and distribution of *A. rubens* is affected by environmental conditions. Knowledge on how these factors affect the observed distribution and abundance of *A. rubens* in the Wadden Sea are necessary to understand the impact and its role on mussel seedbed stability.

7.1.1 The role of salinity and spatial refuge for mussels

A. rubens has been found to able to survive and maintain a viable population at very low and stable salinities (Kowalski 1955). However *Asterias rubens* is an osmoconformer, i.e it has no osmoregulation capacity (Binyon 1961). Osmotic stress caused by salinity changes affects reproduction, eggs and larval development and survival (Saranchova and Flyachinskaya 2001). Adults are also directly affected by salinity changes, which can cause mortality (Figure 7.1, Chapter 3). The larger and the faster a salinity decrease occurs the lower the probability that *A. rubens* survives (Chapter 3).

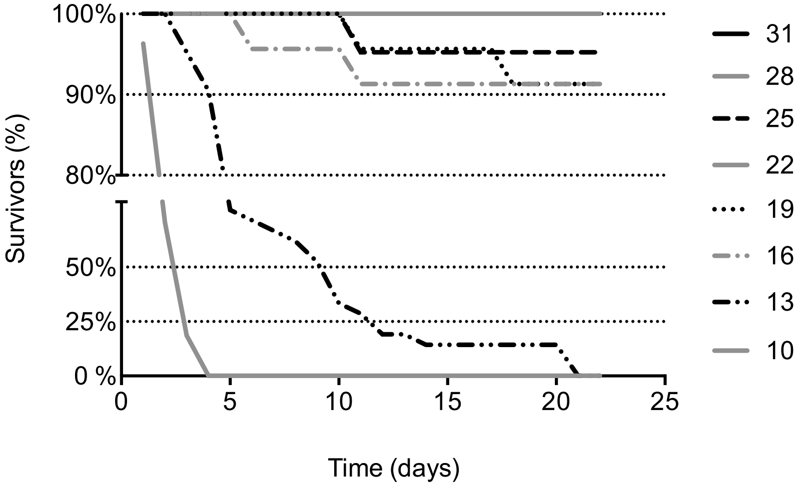


Figure 7.1. Mortality of *A. rubens* after a salinity decrease. Salinity decreased from 31 PSU at a rate of 3 PSU/day for all cases. Mortality accounted in days since aimed salinity was reached.

In the Wadden Sea salinity exhibits large tidal changes that result in a steep salinity gradient from the North Sea to the IJsselmeer sluices, where areas with lower salinity also exhibit the largest tidal range (Duran-Matute et al. 2014) (Figure 7.2). This salinity variability limits the spatial distribution and abundance of *A. rubens* (Figure 7.2, 7.3), probably by preventing settlement or survival of recruits. Seasonal rain and snow melting (Alps) increase the freshwater input in the Wadden Sea resulting in a large decrease of salinity all over the Wadden Sea (Zimmerman 1976) (Figure 7.4). This increase in freshwater input is also reflected in the seasonal abundance of *A. rubens* in the Wadden Sea, that is several times lower after winter (Figure 7.4).

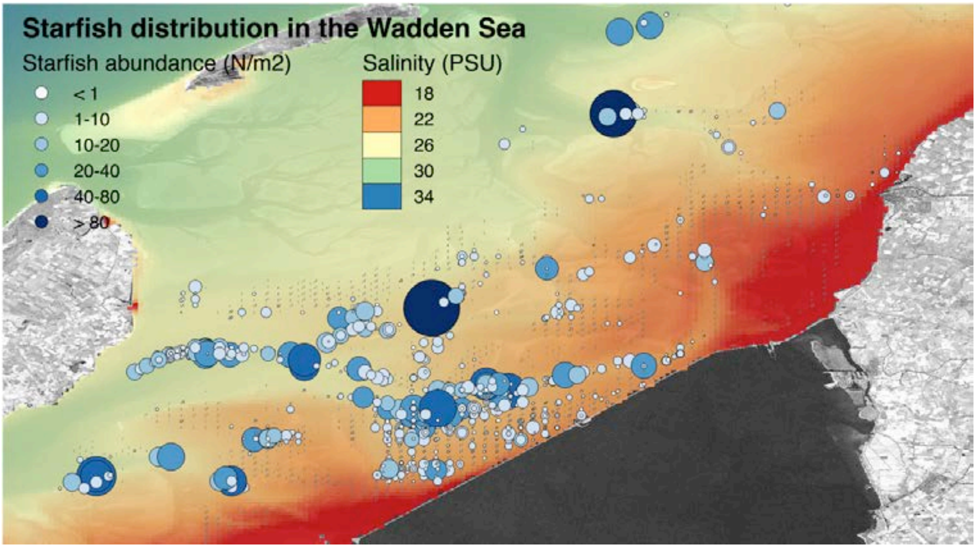


Figure 7.2. Map showing *A. rubens* distribution over the spatial distribution of salinity in the west Dutch Wadden Sea. *A. rubens* sampled during spring (shellfish survey, 1992-2013, IMARES), grey dots represent locations where *A. rubens* has not been recorded. Salinity is the annual mean from Duran-Matute et al. (2014).

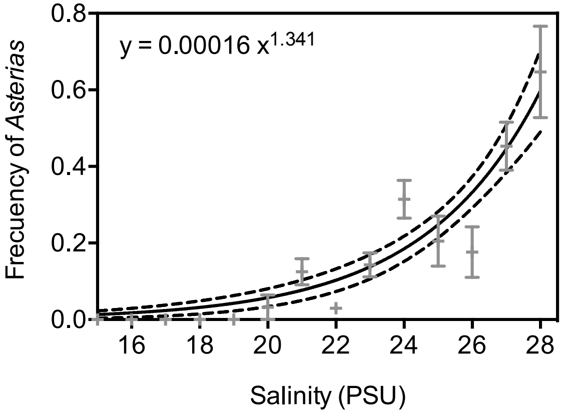


Figure 7.3. Probability of finding *A. rubens* in a sample during autumn plotted against mean annual salinity at the location. Data points are mean ± standard error, n = 664; from Produs 1B monitoring (Ens et al. 2007). Curve represent given regression, slashed lines are the 95% confidence interval. Salinity is the annual mean for the location yielded from the model by Duran-Matute et al. (2014).

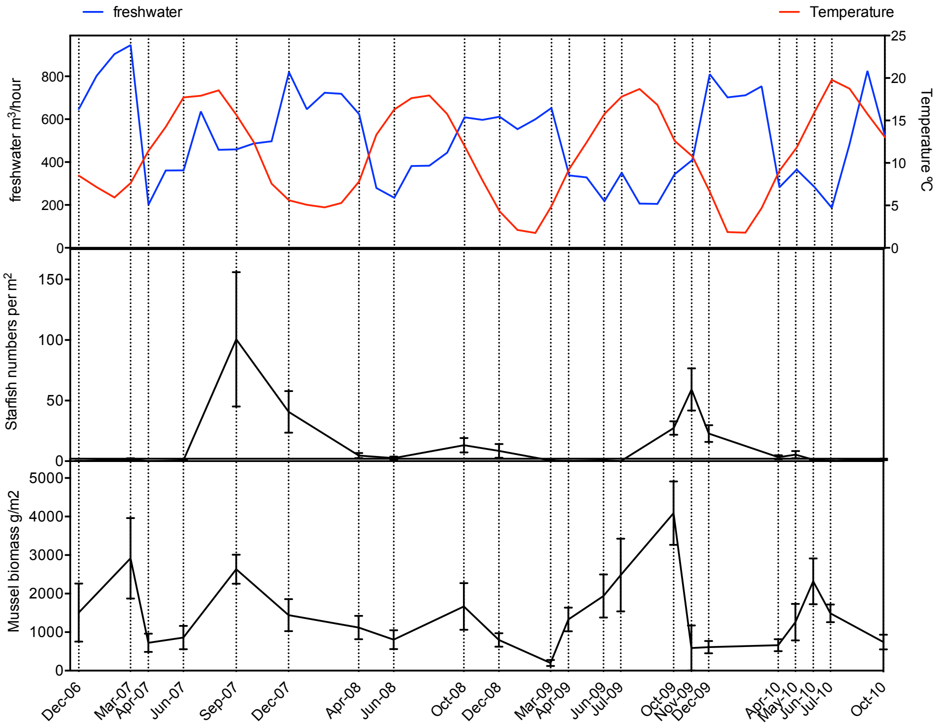


Figure 7.4. Temporal variability of *A. rubens* and mussel abundance Wadden Sea. Top graph shows temperature and freshwater input in the Wadden Sea from the Ijsselmeer (monthly mean, www.live.waterbase.nl). Abundances given as mean ± standard error.

Mussels benefit from the *A. rubens* limitations under salinity changes as they are more resilient to osmotic stress (Saranchova 2001). Mussels are often found in those areas where *A. rubens* is hardly recorded (Ens et al. 2007), areas that coincide with the stability category 1 and category 2 (Figure 1.1). By limiting *A. rubens* distribution and abundance, the salinity regime creates a spatial refuge for mussels. Mussel beds in those refuges are more stable. However salinity in the Wadden Sea depends strongly on freshwater input (anthropogenically controlled), a decrease or increase of the amount of freshwater released in the Wadden Sea will change the salinity gradient and will have an effect on the distribution of *A. rubens.* In that manner during dry years *A. rubens* distribution may be able to reach mussel seedbeds located closer to the freshwater inputs.

7.1.2 Temperature effect on *Asterias rubens* abundance

The spatial effect of temperature is more homogeneous considering the scale of the Wadden Sea (van Aken 2010), and although released freshwater can affect temperature it is not expected to affect the spatial distribution of *A. rubens*. However seasonal changes in temperature affect the predation rate of *A. rubens* on mussels (Chapter 2) regulating the accumulation of reserves necessary for maturation and reproduction. As such cold autumns and winters have an effect on the reproductive output and future recruitment of *A. rubens* (Jangoux and Vloebergh 1973, Jangoux and Impe 1977). Although it is not clear how this may affect the dynamics of *A. rubens* in the Wadden Sea, an effect on recruitment success can be expected which may favour or limit swarms or population blooms. Temperature can also cause *A.rubens* mortality. Ice formation may induce mortality of *A. rubens* or force it to migrate to deeper waters wherever is possible (Gaymer 2001). Additionally temperatures over 20ºC can also cause mortality events (Smith 1940). Therefore extreme seasonal temperatures can affect the abundance and the distribution of *A. rubens.* Hot summers may reduce *A. rubens* abundance and increase stability of mussel beds during autumn and winter. Moreover, cold winters may limit *A. rubens* reproduction and recruitment similarly limiting predation pressure on mussel beds (Guillou et al. 2012).

7.1.3 Hydrodynamics limitations and the link with mussels

Asterias rubens is well adapted to survive under hydrodynamic stress caused by tidal flow and waves when inhabiting hard substrates (Hennebert et al. 2010). However *A. rubens* is poorly adapted to soft sediment where is not able to withstand strong tidal flows (Chapter 4) or storms (Anger et al. 1977, Briggs 1983). In this environment they depend on the capacity of the mussels beds to ameliorate the hydrodynamic stress and provide hard substrate for attachment (Folkard and Gascoigne 2009) (Chapter 4).

The Wadden Sea can experience tidal flows over 1 m s^{-1} and flows over 0.5 m s^{-1} are common in most of the area (Zimmerman 1976, Duran-Matute et al. 2014). Most of the Wadden Sea is comprised of sedimentary bottoms where *A. rubens* is unable to withstand the fast tidal flows present during most of the day unless it is associated with mussels (Chapter 4). This dependency limits the distribution of *A. rubens* within the Wadden Sea to mussel beds (also to oyster beds, piers and other elements that provide a hard substrate) and limits the possibilities for migrating from and to mussel beds unless they are close by or water flow accidentally transport them (Chapter 4).

Asterias rubens dependency on mussels and the limited migration have consequences for the population dynamics of *A. rubens* in the Wadden Sea. *A. rubens* has to settle within mussel beds or be transported accidentally, reducing its capacity to migrate to new feeding grounds once the mussel bed they inhabit is exterminated. Moreover when mussel cover is reduced the *A. rubens* population follows suite.

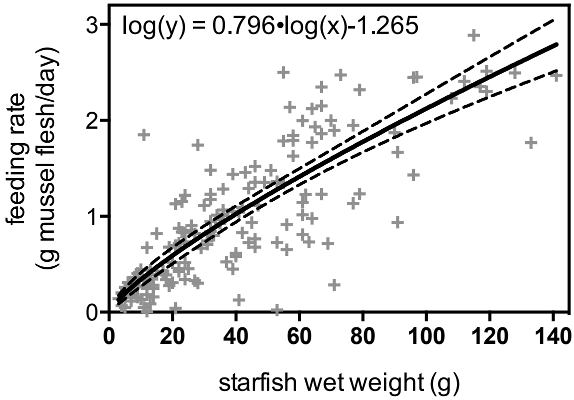


Figure 7.5. *Asterias rubens* feeding rate at size. Feeding rate as mussel flesh (no shell), measured under optimum conditions (15ºC, 31 PSU) in laboratory. Curve represent given regression, slashed lines are the 95% confidence interval.

7.2 The role of environmental conditions on *Asterias rubens* predation performance and growth

Asterias rubens is a voracious predator that exhibits high feeding rates when living under optimal conditions (Chapter 2, 3 and 6) (Figure 7.5). This high feeding rate allows *A. rubens* to grow rapidly and in turn increases its demand for prey (Chapter 3 and 6) (Figure 7.6). However, environmental conditions limit the predation of *A. rubens* imposing physical or physiological limitations to its daily feeding rate, and therefore affecting growth (Chapter 2, 3, 4 and 6). Environmental limitation of *A. rubens* feeding results in an increase of mussel seedbed stability as it reduces the rate at which prey is removed.

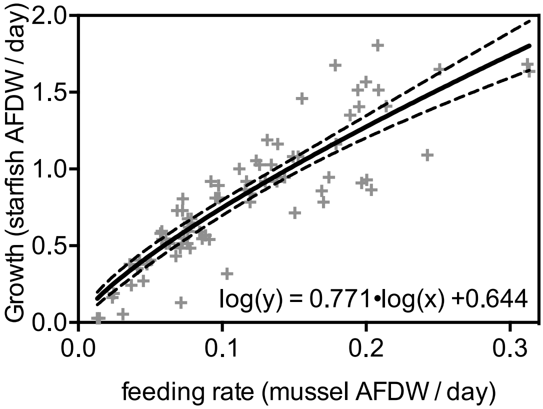


Figure 7.6. *Asterias rubens* growth as a function of food intake. Measured under optimum conditions (15ºC, 31 PSU) in laboratory. Curve represent given regression, slashed lines are the 95% confidence interval.

7.2.1 Seasonal changes in temperature

Temperature has an effect on feeding and therefore on growth. As temperature decreases into the winter season, so does *A. rubens* feeding rate. During cold winters *A. rubens* feeds very little, having little impact on the mussel population (Chapter 2). However during autumn *A. rubens* still exhibits high feeding rates and its impact on the mussel population is higher (Chapter 2). Feeding rate increases rapidly when temperature rises again during spring, however at that time of the year the starfish population has decreased due to salinity stress (Chapter 3), reduction of mussel cover (Chapter 4) and maybe also due to winter ice (Gaymer et al. 2001) (Figure 7.4). Winter temperature affects survival of mussels by affecting *A. rubens* predation rate. As such, mild autumns and winters will result in higher *A. rubens* predation rates during longer time, removing larger amounts of mussels and jeopardizing mussel bed stability. By contrast a cold autumns will result in increased mussel bed stability because predation is reduced.

7.2.2 Salinity effects on feeding rate

A. rubens is able to survive moderate salinity changes (Chapter 3). In those cases, osmotic stress affects the motility of *A. rubens* and reduces its capacity to prey. The larger and the faster the salinity changes the greater the effect on predation rate (Chapter 3). When salinity remains lowered *A. rubens* is able to acclimatize and feeding rate increases with time (Chapter 3). Nonetheless, whether *A. rubens* acclimatized or not to the new salinity, the reduction in feeding activity results in a slower growth (Chapter 3). In a variable environment like the Wadden Sea, the constant changes in salinity with tides and season, means salinity stabilisation does not occur frequently and as such limits the feeding rate of *A. rubens*. The effect of salinity is reflected in individual size (Figure 7.7). Reduced feeding and growth rates mean a reduced impact of *A. rubens*, thereby salinity also has an influence on mussel seedbed stability.

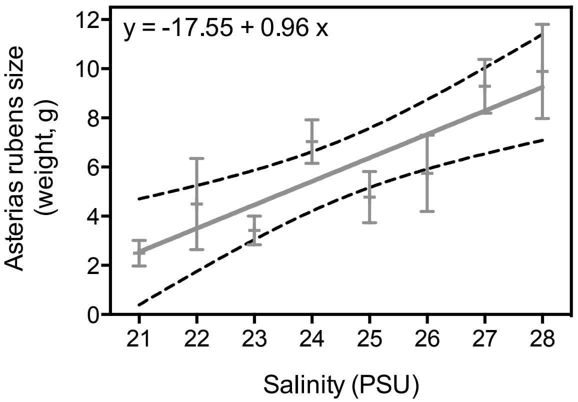


Figure 7.7. Observed size of *A. rubens* during autumn plotted against local salinity. Data points are mean ± standard error, n = 664; from Produs 1B monitoring (Ens et al. 2007). Line represent given regression, slashed lines are the 95% confidence interval. Salinity is the annual mean for the location yielded from the model by Duran-Matute et al. (2014).

7.2.3 Hydrodynamics effects on feeding activity

Asterias rubens depends on the mussels capacity to ameliorate hydrodynamic stress (Folkard and Gascoigne 2009, van Leeuwen et al. 2010), to survive in a dynamic environment (Chapter 4). However even when living within mussel beds, hydrodynamic stress such as results from tidal flows still affects the capacity of *A. rubens* as a predator. *A. rubens* predation activity is reduced when exposed to high velocity flows, independent of the presence of mussels or substrate type (Chapter 4, Figure 4.4). The capacity of mussels to ameliorate hydrodynamic stress depends on their density. Dense mussel beds will reduce the effect of hydrodynamic stress on feeding rate. In most of the Wadden Sea *A. rubens* predation is subject to limitation by tidal flows. However, this limitation changes with mussel density, being of little importance in very dense mussel seedbeds, and becoming an important factor determining predation impact in mussel seedbeds of low density.

7.3 Prey size selection by *Asterias rubens*

7.3.1 Maximum prey size and mussel size refuge

A fully-grown individual of *A. rubens* can prey on the whole size range of blue mussels, including adults and spat. The maximum mussel length that an *A. rubens* individual can prey on is determined by its own size (O'Neill et al. 1983, Norberg and Tedengren 1995). *A. rubens* can prey occasionally on individuals that are bigger than themselves (Figure 7.8). Mussels in the Wadden Sea reach a maximum size of about 60 mm in length, it seems difficult to think that blue mussels can permanently escape predation attaining size refuge by outgrowing a predator that can reach 200 mm in diameter in a few months (Chapter 3, 6), moreover *A. rubens* only needs to be half that size to feed on fully grown mussels (O'Neill et al. 1983). Osmotic stress temporally limits *A. rubens* maximum prey size and forces *A. rubens* to select smaller and easier to handle prey (Chapter 3), however mussels also exhibit a reduced growth under osmotic stress (Almada-Villela 1984), reducing their capacity to escape predation. Reduced *A. rubens* growth during winter, resulting from low temperature may allow mussels to outgrow *A. rubens,* however mussel growth is also considerably reduced during winter due to reduced food availability. There may still be some particular cases where blue mussels may attain temporal or permanent size refuge. *A.rubens* failing to recruit in some areas may allow mussels to attain size refuge for future *A. rubens* settlement, however this may be temporary if new settled individuals find an alternative food source, for example newly settle mussels or barnacles, that allows *A. rubens* recruits to grow large enough to prey older mussels.

7.3.2 Mussel clumping and prey selection by *Asterias rubens*

The bigger the prey the more profitable it may be expected to be. Still *A. rubens* actively selects prey smaller than its maximum capacity in order to avoid damage while predating (Hummel et al. 2011). Prey selection has an effect on prey population dynamics (Chapter 5). However prey population dynamics and behaviour may also have an effect on predator behaviour and prey selection.

Figure 7.8. *Asterias rubens* feeding on a mussel larger than its arm length.

The way that mussels aggregate forming a tri-dimensional matrix has an effect on how predators approach this prey. *A. rubens* prey selection seems to be affected by the spatial organization of mussels. It has been reported that smaller individuals may attain refuge among larger individuals, but only when there is a large enough difference (bimodal length distribution of the mussels in the bed) (Dolmer 1998). The complexity of the attachment among mussels and substrate is an important factor when considering prey selection by *A. rubens*. Our observations show how *A. rubens* stops selecting when mussels form clumps, that behaviour actually results in a greater profit for *A. rubens* (Chapter 5). In the subtidal environment mussels rely on attachment with conspecifics. When an individual is removed the remaining mussels that shared attachments with that individual lost part of their attachment too, as such when individuals are removed from the matrix in the same point, the effort and time required to remove a second one is smaller (wa Kangeri A. K., pers. comm.). This would explain why *A. rubens* stop selecting prey size when mussels form clumps. It also provides insights into why *A. rubens* that concentrates on the edges of a bed, forming feeding fronts are able to successfully and systematically predate the bed (Sloan and Aldridge 1981).

7.4 Determining *Asterias rubens* impact on mussel bed stability

To assess the impact of *A. rubens* on mussel seedbeds it is necessary to assess the growth and predation rate of *A. rubens* when it is affected by environmental conditions. Predation also depends on prey availability, expressed as functional response, which should also be integrated when assessing this impact. The simulation model presented in Chapter 6, integrates the effects of temperature, salinity and hydrodynamics on *A. rubens* predation, and also takes into consideration prey population dynamics. This model can be used as a tool to assess the role of *A. rubens* in the winter stability of mussel beds.

When this model is applied to field observations in the Wadden Sea, obtained between the years 2009 and 2010 (Smaal et al. 2013), the results showed that mussel density decreased in 50% of the cases. In 25% of the cases more than half of the mussel population was removed. Despite environmental limitations *A. rubens* is still able to have an important impact on the mussel bed population.

Environmental conditions change, and it is difficult to clearly describe the effect of each factor in the model output, when all of them change at the same time. Simulations under optimal conditions, or changing only some variables allowed us to understand the effect of each factor considered by the model in the expected mussel bed stability. As expected, predator:prey biomass ratio is the main factor determining the impact of *A. rubens* on mussel seedbed stability (Figure 7.9), specially when no other environmental condition is limiting *A. rubens* performance. Ratios equal to or larger than 0.1 gram of starfish by gram of mussel biomass can completely remove a mussel bed in less than 20 days, under optimal conditions. It is important to consider individual starfish size, as smaller individuals exhibit higher growth and feeding rates per gram having a higher impact (Chapter 6), also mussel biomass is important and even when they share the same predator:prey ratio more dense mussel beds are more resilient to starfish predation (Figure 7.9).

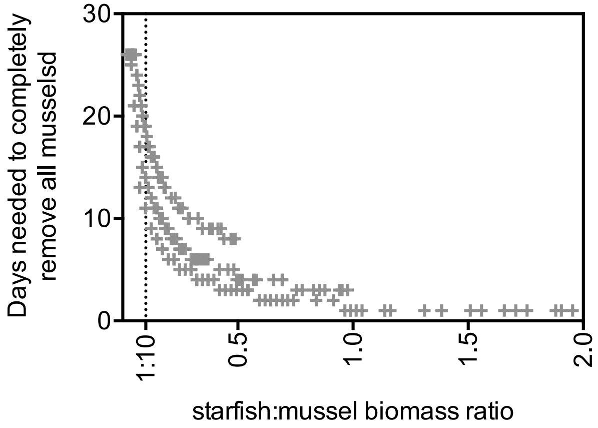


Figure 7.9. Days needed to completely remove all mussel within a mussel bed as a function of the starfish:mussel biomass ration, different curves represent different mussel densities (from top to down 3000, 1500 and 750 g m^{-2}). Model simulation output under optimal conditions (15ºC, 31PSU and 0 m/s). Dotted line shows ratio 1:10 used in the simulations for Figure 7.10.

Although optimal conditions may be encountered in some areas of the mussel distribution (like the Oosterschelde, with a year round constant salinity and reduced tidal amplitude (Smaal and Nienhuis 1992)), that is often not the case in the Wadden Sea. The simulation of a fixed predator:prey ratio (300:3000g predator:prey biomass) along different environmental gradients allowed us to understand the limitations imposed by environmental conditions on the impact of *A. rubens* in mussel seedbed stability (Figure 7.10). In these simulations starfish was never able to completely remove a mussel bed in less than 30 days, besides being present in a biomass ratio able to completely remove a mussel bed in less than 20 days under optimal conditions (Figure 7.9). Simulations predicted only a few cases with a decrease in mussel biomass over 50%. Temperature, salinity and hydrodynamics imposed their own limitations and in almost all cases one or another were limiting starfish predation (for example locations where salinity was higher exhibited higher hydrodynamic conditions that limited starfish predation).

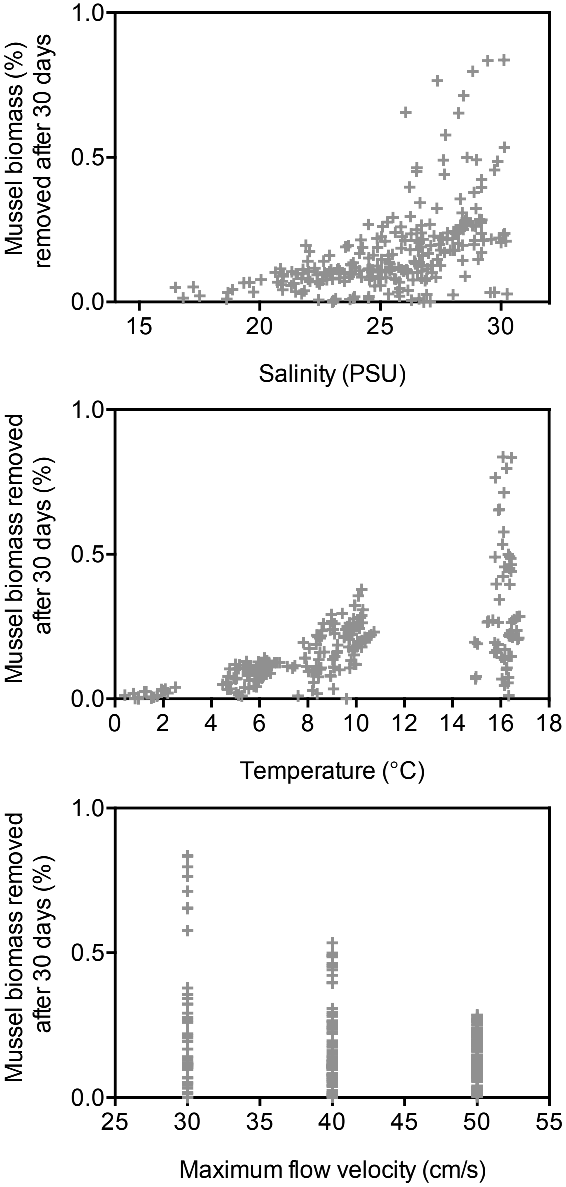


Figure 7. 10. Lost mussel biomass under different environmental conditions (simulation combines all three conditions: temperature, salinity and flow velocity). Ratio is constant 300:3000 (1:10) starfish:mussel biomas (g).

7.5 Conclusions

Subtidal mussel beds are ephemeral structures, most of which disappear shortly after a they come into existence, often due to predation by *A. rubens*. Others may survive for a couple of years, but are continuously receding subject to predation (Glorius et al. 2013). Environmental conditions limit predation and allow mussels to attain a spatial refuge from predation allowing mussel beds to achieve stability. However, environmental conditions in the spatial refuge also affects the mussels that exhibit lower performance due to the salinity regime.

The impact of *A. rubens* is high during autumn and decreases towards winter as temperature decreases (Chapter 2). At the same time rain and snow melting during winter and spring reduce salinity, reducing *A. rubens* abundance and the predation pressure they may exert when temperatures rises in spring (Chapter 3). However, any remaining individuals will keep predating a mussel bed until they finally disappear (Glorius et al. 2013). Salinity is the main driver controlling *A. rubens* dynamics in the Wadden Sea, it limits distribution and abundance and affects predation rate and growth (Chapter 3).

Environmental conditions limit feeding rate and growth of *A. rubens*. However, it is rather improbable for mussels to escape predation by outgrowing starfish maximum prey size. This is due to the fast growth exhibited by *A. rubens* (Chapter 6) and by the fact that adverse environmental conditions also limit mussel growth.

Hydrodynamic conditions in the Wadden Sea are able to completely exclude *A rubens*, which is unable to withstand fast flow when inhabiting soft bottoms. Under these conditions mussels may avoid predation by *A. rubens.* However, the association of mussels in clumps provides *A. rubens* with shelter and a hard substrate damping the effect of hydrodynamic stress, allowing *A. rubens* to persist within mussel beds in the Wadden Sea despite unstable sediments (Chapter 4).

Starfish can destroy mussel seedbeds before winter, however their capacity to do so is strongly affected by environmental conditions, which affect their predation efficiency and distribution. As such the simple presence of *A. rubens* on a bed is insufficient basis for predicting bed stability. Morevover, as other predators do, moderate predation pressure by starfish can be beneficial for mussel seedbed survival as it may ease intraspecific competition, a factor that causes important mortality in mussel juvenile stages (Petraitis 1995). We recommend that future management decision employ the new insights and tools provided here to aid in deciding the fate of wild seedbeds.

The new insights provided in this thesis further support the understanding of predation by *A. rubens* and enables both the shellfish culture industry and environmental policy makers to take measures that may help reduce the impact this predator has on mussel beds. The model provided in the chapter 6, represents a tool for the effective management of *A. rubens* impact on mussel productivity particularly when determining mussel bed stability of doubtful cases (Chapter 1).

Despite the knowledge gathered here it is important to consider that knowledge on the reproduction, egg and larval development and transport, settlement timing, and how this is related to the settlement of mussels or other organisms serving as alternative food for *A. rubens* is still limited. More research is warranted to understand *A. rubens* spatial and temporal distribution including the formation of swarms and feeding fronts if we are to assess its impact on shellfish populations.

References

Agüera, A., M. Trommelen, F. Burrows, J. M. Jansen, T. Schellekens, and A. Smaal. 2012. Winter feeding activity of the common starfish (*Asterias rubens* L.): The role of temperature and shading. Journal of Sea Research 72:106–112.

Almada-Villela, P. C. 1984. The effects of reduced salinity on the shell growth of small *Mytilus edulis*. Journal Of The Marine Biological Association Of The United Kingdom 64:171–182.

Anger, K., U. Rogal, G. Schriever, and C. Valentin. 1977. In-situ investigations on the echinoderm *Asterias rubens* as a predator of soft-bottom communities in the western Baltic Sea. Helgoländer Wissenschaftliche Meeresuntersuchungen 29:439–459.

Barbeau, M. A., B. G. Hatcher, R. E. Scheibling, A. W. Hennigar, L. H. Taylor, and A. C. Risk. 1996. Dynamics of juvenile sea scallop (Placopecten magellanicus) and their predators in bottom seeding trials in Lunenburg Bay, Nova Scotia. Canadian Journal of Fisheries and Aquatic Sciences 53:2494–2512.

Binyon, J. 1961. Salinity Tolerance and Permeability to Water of the Starfish *Asterias rubens* L. Journal Of The Marine Biological Association Of The United Kingdom 41:161–174.

Briggs, C. F. 1983, February. A study of some sublittoral populations of Asterias rubens (L.) and their prey. Port Erin. Ph.D thesis. Liverpool University

Dolmer, P. 1998. The interactions between bed structure of *Mytilus edulis* L. and the predator *Asterias rubens* L. The Journal of Experimental Marine Biology and Ecology 228:137–150.

Duran-Matute, M., T. Gerkema, G. J. de Boer, J. J. Nauw, and U. Gräwe. 2014. Residual circulation and fresh-water transport in the Dutch Wadden Sea: a numerical modeling study. Ocean Science Discussions 11:197–257.

Ens, B. J., J. Craeymeersch, F. Fey-Hofstede, H. J. L. Heessen, A. C. Smaal, A. G. Brinkman, R. Dekker, J. Van Der Meer, and M. V. Stralen. 2007. Sublitorale natuurwaarden in de Waddenzee. Wageningen IMARES. C077/07

Folkard, A. M., and J. C. Gascoigne. 2009. Hydrodynamics of discontinuous mussel beds: Laboratory flume simulations. Journal of Sea Research 62:250–257.

Gallagher, T., C. A. Richardson, R. Seed, and T. Jones. 2008. The Seasonal movement and abundance of the starfish *Asterias rubens* in relation to mussel farming practive: a case study from the Menai Strait, UK. Journal of Shellfish Research 27:1209–1215.

Gaymer, C. F. 2001. Use of prey resources by the seastars Leptasterias polaris and *Asterias vulgaris*: a comparison between field observations and laboratory experiments. The Journal of Experimental Marine Biology and Ecology 262:13–30.

Gaymer, C. F., J. H. Himmelman, and L. E. Johnson. 2001. Distribution and feeding ecology of the seastars Leptasterias polaris and Asterias vulgaris in the northern Gulf of St Lawrence, Canada. Journal Of The Marine Biological Association Of The United Kingdom 81:827–843.

Glorius, S., A. Rippen, M. de Jong, B. van der Weide, J. Cuperus, A. Bakker, and M. van Hoppe. 2013. De ontwikkeling van niet beviste sublitorale mosselbanken 2009-2012. Page 56. 2012 edition. IMARES Wageningen UR, Den Helder. C199.13

Hancock, D. A. 1955. The Feeding Behaviour of Starfish on Essex Oyster beds. Journal Of The Marine Biological Association Of The United Kingdom 34:313–331.

Hennebert, E., D. Haesaerts, P. Dubois, and P. Flammang. 2010. Evaluation of the different forces brought into play during tube foot activities in sea stars. The Journal of Experimental Biology 213:1162–1174.

Hummel, C., P. Honkoop, and J. van der Meer. 2011. Small is profitable: No support for the optimal foraging theory in sea stars *Asterias rubens* foraging on the blue edible mussel *Mytilus edulis*. Estuarine, Coastal and Shelf Science 94:89–92.

Jangoux, M., and E. V. Impe. 1977. The annual pyloric cycle of *Asterias rubens* L. (ECHINODERMATA: ASTEROIDEA). The Journal of Experimental Marine Biology and Ecology 30:165–184.

Jangoux, M., and M. Vloebergh. 1973. Contribution á l'étude du cycle annuel de reproduction d‘une population d'*Asterias rubens* (Echinodermata, Asteroidea) du littoral Belge. Netherlands Journal of Sea Research 6:389–408.

Kowalski, R. 1955. Untersuchungen zur Biologie des Seesternes Asterias rubens L. in Brackwasser. Kieler Meeresforschungen XI.

Menge, B. A. 1982. Effects of feeding on the environment: Asteroidea. Pages 521–551 *in* M. Jangoux, J. M. Lawrence, and J. M. Lawrence, editors. Echinoderm Nutrition. A.A. Balkema, Rotterdam.

Norberg, J., and M. Tedengren. 1995. Attack behaviour and predatory success of *Asterias rubens* L. related to differences in size and morphology of the prey mussel *Mytilus edulis* L. The Journal of Experimental Marine Biology and Ecology 186:207–220.

O'Neill, S., A. Sutterlin, and D. Aggett. 1983. The effects of size-selective feeding by starfish (*Asterias* *vulgaris*) on the production of mussels (*Mytilus edulis*) cultured on nets. Aquaculture 35:211–220.

Petraitis, P. S. 1995. The Role of Growth in Maintaining Spatial Dominance by Mussels (*Mytilus edulis*). Ecology 76:1337–1346.

Saranchova, O. L. 2001. Research into tolerance for the environment salinity in sea starfish *Asterias rubens* L. from populations of the White Sea and Barentz Sea. The Journal of Experimental Marine Biology and Ecology 264:15–28.

Saranchova, O. L., and L. P. Flyachinskaya. 2001. The Influence of Salinity on Early Ontogeny of the Mussel *Mytilus edulis* and the Starfish *Asterias rubens* from the White Sea. Russian Journal of Marine Biology 27:87–93.

Sloan, N. A., and T. H. Aldridge. 1981. Observations on an aggregation of the starfish *Asterias rubens* L. in Morecambe Bay, Lancashire, England. Journal of Natural History 15:407–418.

Smaal, A. C. 2002. European mussel cultivation along the Atlantic coast : production status , problems and perspectives. Hydrobiologia 484:89–98.

Smaal, A. C., A. G. Brinkman, T. Schellekens, J. M. Jansen, A. Agüera, and M. R. van Stralen. 2014. Ontwikkeling en stabiliteit van sublitorale mosselbanken. Page 32. IMARES Wageningen UR, Yerseke.

Smaal, A. C., and P. H. Nienhuis. 1992. The eastern Scheldt (The Netherlands), from an estuary to a tidal bay: A review of responses at the ecosystem level. Netherlands Journal of Sea Research 30:161–173.

Smith, G. F. M. 1940. Factors limiting distribution and size in the starfish. Journal of the Fisheries Research Board of Canada 5:84–103.

van Aken, H. M. 2010. Meteorological forcing of long-term temperature variations of the Dutch coastal waters. Journal of Sea Research 63:143–151.

van Leeuwen, B., D. C. M. Augustijn, B. K. van Wesenbeeck, S. J. M. H. Hulscher, and M. B. De Vries. 2010. Modeling the influence of a young mussel bed on fine sediment dynamics on an intertidal flat in the Wadden Sea. Ecological Engineering 36:145–153.

Witman, J. D., S. J. Genovese, J. F. Bruno, J. W. McLaughlin, and B. I. Pavlin. 2003. Massive Prey Recruitment and the Control of Rocky Subtidal Communities on Large Spatial Scales. Ecological Monographs 73:441–462.

Zimmerman, J. T. F. 1976. Mixing and flushing of tidal embayments in the western Dutch Wadden Sea. Part I: Distribution of Salinity and calculation of mixing time scales. Netherlands Journal of Sea Research 10:149–191.

Appendices

Summary

Samenvatting

Sumario

Acknowledgements

Curriculum vitae

WIAS TSP

Summary

Mussel beds are an important ecological component in the Wadden Sea. Mussels' offspring settle massively in new suitable areas, forming seedbeds that may disappear again within months. The probability of a seedbed to survive the first winter is defined as seedbed stability; a definition that plays a very important role in the management of newly settled seedbeds. Many factors are important in the survival or extinction of seedbeds. Predation is thought to be particularly important during the first year after settlement and therefore key to survival. Many predators feed on mussel beds, but for most of them the potential to exterminate a seedbed is restricted by different factors such as prey selection or competition. Common starfish (*Asterias rubens*) are capable of concentrating/aggregating in high densities on mussel seedbeds making them an especially important factor limiting/affecting survival of mussel seedbeds. This study assesses the capacities of starfish as a mussel seed predator. It also provides tools and information to assess the risks of a seedbed being attacked and exterminated by starfish.

The first objective of this work was to quantify the effect of an environment with large salinity variations, strong tidal currents and drastic seasonal temperature changes on the predation performance of *A. rubens.* In **Chapter 2** the role of temperature and shading on winter predation was studied. The results showed that temperature limits feeding rate and feeding activity of starfish during winter. However, starfish feeding rate exhibited very high sensitivity to temperature changes. Light intensity affected both feeding rate and feeding activity. We conclude that starfish may not be an important factor destabilizing seedbeds during the average winter, but its importance may grow along with the increasing mean winter temperature due to climate change.

In **Chapter 3** the impact of salinity changes on predation performance and survival was assessed. Salinity is the main driver of species distributions in the Wadden Sea. Results show that salinity affected predation performance by reducing feeding activity and causing changes in prey selection. Moreover, as acclimation occurred, *A. rubens* predation performance improved in all treatments with survivors. We conclude that osmotic stress due to a salinity decreases determines *A. rubens* distribution,

abundance and potential impact on the prey population. However this effect is influenced by the magnitude of the change in salinity and its timescale.

Hydrodynamic stress due to strong tidal currents is an important factor determining the activity of mobile predators. Importantly, mussels, as recognized ecosystem engineers, can change their environment by, among other, ameliorating hydrodynamic stress. In **Chapter 4** the effect of tidal currents on predation rate was assessed, however, the chapter also tackles the role of hydrodynamic stress amelioration by mussels on the *A. rubens* population. The results suggest that mussels interact with their own predator beyond the role of food source, by ameliorating environmental stress, creating an additional dependence link between the foundation species and the predator, which potentially has major implications for ecosystem structure and stability.

Having assessed the effects of the environment, this thesis goes on to expand on the knowledge of mussel - starfish interaction. Prey selection affects prey population dynamics. At the same time, pray selection can be affected by prey population dynamics and behaviour. In **Chapter 5**, we assessed the role of mussel association with conspecifics at high densities on prey selection by *A. rubens*. We concluded that size selection does not always lead to an improvement in net profit. Size selection is a trade-off between energy yield and predation energy costs, which is affected by prey behaviour. As such, under certain circumstances, increasing prey size does not result in an improvement of net profit for the predator. We discussed the effects of this behaviour on the predator-prey dynamics and the mussel culture industry.

The results of the prior chapters were integrated in **Chapter 6** with field observations and literature to develop a simulation model. This model was designed to simulate growth of mussels and starfish, predation by starfish and mussel mortality. This model intended to provide a tool for the fishery and conservation management programmes. The resulting model is able to determine predation by starfish in the short term. This information may then be used to assess potential losses in productivity, survival of natural seedbeds, etc. It can also be used to predict the likely effect of future environmental change scenarios on the potential impact of *A. rubens* on this important resource.

In the general discussion, **Chapter 7**, previous literature, field data and the results from this thesis are summarised and reviewed to explain the spatial distribution of *A. rubens* in the Wadden Sea and the role of environmental conditions on *A. rubens* predation rate. Model simulations are used to answer the question: **What is the role of *A. rubens* predation in mussel seedbed stability?**

Samenvatting

Mosselbanken zijn een belangrijke ecologische component in de Waddenzee. Nakomelingen van mosselen vestigen zich massaal in nieuwe geschikte gebieden en vormen daarmee zogenoemde, zaadbanken. Deze banken kunnen, omwille verschillende factoren, , binnen enkele maanden weer verdwijnen. De kans dat een zaadbank zijn de eerste winter overleeft wordt gedefinieerd als zaadbank stabiliteit. Ze definitie speelt een zeer belangrijke rol in het beheer van nieuw gevestigde zaadbanken. Veel factoren zijn van belang bij het overleven of uitsterven van zaadbanken. Met name predatie wordt geacht van belang te zijn in het eerste jaar na de broedval , en is daarom cruciaal voor de overleving van een bank. Veel roofdieren voeden zich van mosselbanken, maar voor de meeste wordt het potentieel van uitroeien van een zaadbank beperkt door verschillende factoren zoals prooi selectie of concurrentie. Zeesterren (*Asterias rubens*) zijn in staat om zich te concentreren grote dichtheden op mossel zaadbanken waardoor ze een belangrijke beperkende invloed kunnen hebben op de overleving van een mossel zaadbank. Deze studie beoordeelt de capaciteiten van zeester als mosselzaad roofdier. Het biedt ook de nodige kennis en werktuig het risico van een aanval en uitroeiing door zeesterren te beoordelen.

De eerste doelstelling van dit werk was om het effect van dynamische omgevingen variabelen, zoals grote variaties in zoutgehalte, sterke getijdenstroming en drastische seizoensgebonden temperatuur veranderingen, op de predatie prestaties van *A. rubens* kwantificeren. In **hoofdstuk 2** wordt de rol van de temperatuur en schaduw op winter predatie bestudeerd. De resultaten toonden aan dat de predatie snelheid en bewegingen activiteit van de zeester beperkt worden door temperatuur in de winter. Echter, zeester predatie snelheid toonde een zeer hoge gevoeligheid voorveranderingen in de temperatuur. Verder wordt er aangetoond dat lichtintensiteit zowel predatie snelheid als voedselopname bij invloed. Geconcludeerd wordt dat het belang van zeesterren in de destabilisatie van zaadbanken gedurende een huidig gemiddelde winter niet zoo groot is, maar dat deze snel kan groeien met de stijgende temperatuur als gevolg van klimaatverandering.

In **hoofdstuk 3** wordt de rol van zoutgehalte veranderingen op predatie prestaties en overleving beoordeeld. De zoutgehalte is de belangrijkste factor in de verspreiding van soorten in de Waddenzee. Resultaten laten zien dat de prestaties van zeesterren beïnvloed worden door zoutgehalte, waarbij er een vermindering van voedselopname en een verandering in de prooi selectie plaats vind bij verandering in zoutgehalte. Bovendien, als *A. rubens* kan acclimatiseren, dan verbeteren de predatie prestaties ook, behalve, in dodelijke behandelingen. We concludeerden dat osmotische stress als gevolg van een afname van het zoutgehalte de verspreiding van en mogelijke impact op de prooi bevolking door *A. rubens* bepaalt. Dit effect wordt sterk beïnvloed door de grootte van de veranderingen in zoutgehalte en de tijdschaal waarop het plaats vind.

Hydrodynamische stress als resultaat van sterke getijdestromingen zijn een belangrijke factor in de activiteit van de mobiele zeesterren. Als erkende ecosysteem-ingenieur kunnen mosselen een grote invloed hebben op hun omgeving waaronder het remmen van stroming en beperken van hydrodynamisch stress. In **hoofdstuk 4** wordt de rol van de getijdenstroming in predatie vastgesteld. Het hoofdstuk richt zich ook op de rol van mosselen in hydrodynamische stress-verbetering voor de *A. rubens* populatie. De resultaten suggereren dat de mossel meer dan alleen de rol van voedsel vervult voor zijn roofdier. Door het beperken omgevingen stress ontstaat er een extra afhankelijkheden verband tussen de prooi soort en de predator. Deze samenhang heeft potentieel grote gevolgen voor de structuur en stabiliteit van het ecosysteem.

Volgend op de vergaarde kennis betreffende de gevolgen van het milieu op zeester predatie werd het werk van dit proefschrift gericht op uitbreiding van de kennis over de interactie tussen mosselen en zeesterren. Hierin word duidelijk dat prooi selectie prooi populatiedynamiek kan beïnvloeden, en tegelijkertijd kan prooi populatiedynamiek en gedrag prooi selectie bij invloeden. In **hoofdstuk 5** onderzochten we de invloed van mossel omgang met soortgenoten bij hoge dichtheden in prooi selectie door A. rubens. We concluderen dat de prooi grootte selectie niet altijd leidt tot een verbetering van de netto energie winst. Prooi grootte selectie is een evenwichtige wisselwerking tussen energieopbrengst en energiekosten van predatie, waarbij het laatste wordt beïnvloed door prooi gedrag. Als zodanig, leidt onder bepaalde omstandigheden, toenemende prooi omvang niet tot een verbetering van de nettowinst voor het roofdier. We bespreken de gevolgen van dit prooi gedrag op de predator-prooi dynamiek en de mosselkweek industrie.

De resultaten van de voorgaande hoofdstukken worden in **hoofdstuk 6** samengebracht met veldwaarnemingen en literatuur om een simulatiemodel te ontwikkelen wat in staat is om de groei van mosselen en zeesterren, predatie door zeesterren en mossel sterfte te simuleren. Dit model is erop gericht om een instrument te bieden voor zowel de visserij als behouden programma's. Het resulterende model is in staat om korte termijnen voorspellingen te maken voor zeester. Deze informatie kan worden gebruikt om bijvoorbeeld potentiele verliezen in productiviteit te beoordelen of overleving van natuurlijke zaadbanken te bepalen. Het kan ook gebruikt worden om de impact van *A. rubens* op de mossel populatie bij toekomstige veranderingen in het milieu te voorspellen.

In de algemene discussie, **hoofdstuk 7**, wordt bestaande literatuur, veldgegevens en de resultaten van dit proefschrift samengevat en beoordeeld om de ruimtelijke verdeling van *A. rubens* in de Waddenzee uit te leggen. De rol van omgevingsfactoren in *A. rubens* predatie wordt hierin ook besproken. Modelsimulaties worden gebruikt om de toegepaste vraag, “**Wat is de rol van A. rubens predatie in mossel zaadbank stabiliteit?**” te beantwoorden.

Sumario

Los bancos de mejillones son un importante componente ecológico en el mar de Wadden. Los mejillones reclutan de forma masiva y se asientan formando nuevos bancos de juveniles (semillas) que pueden desaparecer al poco tiempo. La probabilidad de estos bancos de sobrevivir a su primer invierno se define como estabilidad y viene dada por varios factores. Entre estos factores destaca el impacto de depredadores. Varias especies son capaces de consumir importantes cantidades de semillas de mejillón durante su primer año de existencia, siendo un factor de importancia para la supervivencia de estos bancos. Existen muchos depredadores que se alimentan de semillas de mejillón, pero la mayoría carece del potencial para eliminar completamente los bancos de mejillones debido a factores como competición con otros predadores o porque sólo se alimentan de mejillones con unas características particulares de tamaño, desarrollo, etcétera. La estrella de mar común, *Asterias rubens* L., aparece a menudo formando concentraciones con un gran numero de individuos, lo que la convierte en un depredador importante que es capaz de limitar o determinar la supervivencia de los bancos de semilla de mejillón. El presente trabajo proporciona herramientas e información para calcular el riesgo que representa *A. rubens* para la supervivencia de los bancos de semillas.

El primer objetivo de este estudio ha sido cuantificar la capacidad de *A. rubens* de consumir mejillones expuesta a las duras condiciones abióticas en el mar de Wadden. Este mar es en realidad un estuario y se caracteriza por presentar grandes gradientes de salinidad, fuertes corrientes y drásticos cambios de temperatura con los cambios de estación. En el **Capítulo 2** de esta tesis se estudia el rol de la temperatura y de la intensidad de luz en la reducción del consumo de mejillones por *A. rubens* que se observa durante el invierno. La cantidad de mejillones consumidos por *A. rubens* cambia rápidamente con la temperatura. La intensidad de la luz también afecta la actividad de *A. rubens*. En este capítulo concluimos que el consumo de mejillones por *A. rubens* no es un factor importante en la supervivencia de mejillones durante el invierno. Aunque esta situación puede cambiar con el incremento de temperatura debido al calentamiento global, o en caso de otoños e inviernos de temperaturas suaves.

El **Capítulo 3** se centra en el efecto de los cambios de salinidad en la capacidad de *A. rubens* de consumir mejillones, al igual que se observa como estos cambios de salinidad pueden producir mortalidad en la población de estrellas de mar. Los resultados de este capítulo muestran como los cambios de salinidad afectan el rendimiento y la actividad de *A. rubens*, que no sobrevive en los casos más extremos. Los cambios de salinidad afectan la distribución, abundancia y actividad de *A. rubens*, aunque la magnitud de este efecto depende de cómo de grande y rápido sea el cambio de salinidad.

El estrés ocasionado por las condiciones hidrodinámicas del medio es un factor importante que afecta especialmente la distribución de predadores móviles. Los mejillones tienen la capacidad de cambiar estas condiciones hidrodinámicas y reducir el estrés que provoca. El **Capítulo 4** estudia el rol de las corrientes producidas por los cambios de marea en la actividad de *A. rubens* considerando la capacidad de los mejillones de aliviar el estrés producido estas corrientes. Los resultados sugieren que los mejillones son algo más que comida para *A. rubens,* porque proporcionan protección. Este dependencia de *A. rubens* hacia los mejillones tienen importantes implicaciones en la estructura y la estabilidad del ecosistema.

Una vez los efectos del medio han sido determinados, el trabajo de esta tesis se enfoca a ampliar conocimientos sobre la interacción predador-presa entre el mejillón y *A. rubens*. Cuando un predador prefiere una presa en particular sobre las demás, produce cambios en la dinámica de poblaciones de la presa. Al mismo tiempo el que el predador elija una presa sobre otras puede estar determinado por el comportamiento de la presa o su abundancia. El **Capítulo 5** describe el rol que tiene la asociación de mejillones entre ellos en las individuos consumidos por *A. rubens*. Este estudio concluye que el mejillón elegido para consumo no siempre tiene que ser el mejor o mas nutritivo. La elección de presa es el resultado de un equilibrio entre la energía obtenida de la presa y la energía necesaria para obtener y manejar la presa, esto último es a su vez una consecuencia del comportamiento de la presa que intenta no ser consumida. La elección de *A. rubens* de unos individuos de mejillón sobre otros tiene consecuencias en la dinámica de poblaciones del mejillón y su cultivo.

Los resultados de los capítulos previos se han integrado con observaciones de campo y resultados de otros estudios son usados en el **Capítulo 6** para desarrollar un modelo que simule el crecimiento de mejillones y *A. rubens* considerando depredación por *A. rubens* y otros factores que determinan la supervivencia de los mejillones. Este modelo tiene como objetivo ser capaz de simular el impacto de *A. rubens* en la supervivencia de los bancos de mejillones naturales, en cultivos y en los colectores. También puede ser usado para predecir o aproximar futuros escenarios medio ambientales en el rol de *A. rubens* en la estabilidad de los bancos de mejillones.

En la discusión general, **Capítulo 7**, resultados previos, observaciones de campo y los resultados de esta tesis son revisados para describir la dinámica de poblaciones de *A. rubens* en el mar de Wadden considerando el rol de las condiciones ambientales en su actividad. Simulaciones usando el modelo desarrollado en el capítulo 6 son usadas para dar respuesta al problema considerado en esta tesis: **¿Cuál es el rol de *A. rubens* en la estabilidad de los bancos de semillas de mejillón?**

Acknowledgements

I am extremely grateful to many people that have supported me during the past four years and that have contributed in one way or another to this thesis.

I am especially grateful to Prof. Aad Smaal and Dr. Jeroen Jansen for giving me the opportunity to do this PhD. And giving me freedom to develop my ideas and research, while keeping an eye on me.

Prof. Aad Smaal, the promoter of this PhD, has busy schedule. However he was there when needed, taking an indispensable leading role during the last months.

Dr. Jeroen Jansen, as a daily supervisor. He gave me unconditional support to develop my own ideas and transmitted to me his ambitious research character, teaching me to have confidence in my skills. We had many long discussions about a vast array of scientific topics not always concerning this thesis.

Dr. Tim Schellekens, also a daily supervisor, had a very important role on the completion of this PhD. He offered a different perspective for my research that lead to improved results and discussion. He always had something to say, there was always something that could be explained better or done in a better way. Discussions with him always led to great improvement of manuscripts or to new ideas to analyse experimental or field data.

Besides having a good and complete supervision team, I always sought for expertise outside the confidence zone. This led me to meet and collaborate with great researchers from different countries and research areas that enriched this thesis with their contributions. I want to thank all of them. To Dr Monique Guillou from Brest University for sharing her knowledge on *Asterias* when a complete stranger approached her by email. Dr. Tjeerd Bouma from NIOZ provided me with support from experimental design until submission on the hydrodynamics chapter. I had several discussions on starfish with Prof. Johan van de Koppel, who also helped with the hydrodynamic experiments and chapter. I have to thank also Michel Trommelen and

Frances Burrows, their work provided me with valuable data to have a great start that made my work easier during this four years.

While working for IMARES, I have the opportunity of meeting many colleagues as I moved from one location to the other. Many people have contributed to this thesis work by helping me with laboratory experiments and field sampling. To Ad van Gool, Eva Hartog (from IMARES Yerseke) and Arnold Bakker (IMARES Den Helder) for their help sourcing starfish and mussels for the experiments from fishermen, buoys or any other imaginable source. To Andrea, Lilian, Babeth, Wilma, Erika and Anneke from IMARES Den Helder, for their help in the laboratory when I most needed.

Funding for this research was provided by the Dutch Ministry of Economy, Agriculture and Innovation and by the Producers Organisation of Dutch Musselculture through the PRODUS project and from the Dutch Waddenfonds, Dutch Ministry of Instrastructure and Enviornment (Rijkswaterstaat) via the project Mosselwad.

I shared these four years with several fellow PhD students. I was lucky having them around, always helpful and open to discussions with more or less scientific content, often to release stress. To Jacob, Wouter, Brenda, Isabel and Arno thanks for the company, for showing me how things worked and for the help when I was lost in translation or on bureaucracy. To Arno and Jen thanks for taking all the time to check and correct the spelling and grammar of my manuscripts when you yourself have so much work to do.

I am really grateful to all, old and new friends, for being there to support me during these four years.

Escribo estas líneas en castellano para agradecer a mis hermanos y a mis padres todo el apoyo que me han dado de manera totalmente incondicional durante todos estos años. A pesar de no entender exactamente que es lo que hago ni adonde me llevará en el futuro. Muchas gracias, sin vosotros nunca habría sido posible llegar hasta aquí y completar este Ph.D.

About the author

Antonio Agüera was born on May 12th, 1982 in Málaga (Spain) where he grew up and lived until graduating from I.E.S Santa Rosa de Lima (secondary school) in 2000. He studied Marine Science at the University of Cadiz in Puerto Real (Cádiz, Spain) where he graduated in 2005 and obtained a Diploma (D.E.A.) in Marine Zoology in 2008. In that same year he moved to Ireland to pursue a research masters on aquatic biology and fisheries supervised by Dr. Deirdre Brophy at the Galway-Mayo Institute of Technology (Galway, Ireland) until 2010. A month before graduating, he started his PhD research on starfish predation and mussel bed ecology at Wageningen IMARES (Yerseke, The Netherlands) supervised by Dr. Jeroen Jansen, Dr. Tim Schellekens and Prof. Aad Smaal. After completion of his PhD he started to work as a postdoctoral researcher at the Marine Biology Laboratory at Université Libre de Bruxelles (Brussels, Belgium).

Peer reviewed publications

2012 Agüera, A., Trommelen, M., Burrows, F., Schellekens, T., Jansen, J.M., Smaal, A. C. (2012). Winter Feeding activity of the common starfish (*Asterias rubens* L.): The role of temperature and shading. Journal of Sea Research, 72:106-112

Agüera, A., Brophy, D. (2012). Growth and age of Atlantic saury, *Scomberesox saurus saurus* (Walbaum), in the Northeastern Atlantic Ocean. Fisheries Research, 131:60-66.

2011 Agüera, A., Brophy, D. (2011). Use of saggital otolith shape analysis to discriminate Northeast Atlantic and Western Mediterranean stocks of Atlantic saury, *Scomberesox saurus saurus* (Walbaum). Fisheries Research 110:465-471.

2008 Agüera, A. Oliver, P.G. (2008). Species discrimination in seven species of *Barbatia* (Bivaliva: Arcoidea) from Thailand with a redescription of *B. grayana* (Dunker, 1858). The Raffles Bulleting of Zoology, Suppl. 18: 7-23.

WIAS Graduate School Training and Supervision Program

Education and training 2010-2014	**ECTS**
The basis package	**3.0**
WIAS Introduction Course	
Ethics and philosophy of animal sciences	
Conferences, seminar and presentations	**11**
ECClima 2011, Porto, Portugal. (poster presentation)	
14th International Conference in Shellfish restoration, Stiriling, UK (poster presentation)	
41st Benthic Ecology Meeting, Norfolk, USA. (oral presentation)	
14th International Echinoderm Conference, Brussels, Belgium (oral & poster presentations)	
European Marine Biology Symposium 2013, Galway, Ireland (poster presentation)	
43rd Benthic Ecology Meeting, Jacksonville, USA (oral presentation)	
Disciplinary and Interdisciplinary courses	**11**
Spatial Ecology (SENSE)	
Dynamic Energy Budget (DEB) model	
Zero inflated models and GLMM with R	
Professional Skills	**19**
High Impact writing in Science	
Ecological modelling and C++ programming	
Preparing own PhD research proposal	
External training period at Aarhus University	
Didactic skills	**1**
Supervision of HBO thesis: “Functional response of starfish (*Asterias rubens*) preying on mussels (*Mytilus edulis*). Frank Brouwer 2012, HAS Den Bosch.	
Total	**45**