Canadian Journal of Fisheries and Aquatic Sciences
Journal canadien des sciences halieutiques et aquatiques

The influence of commercial harvesting on parasite infection in the bivalve *Austrovenus stutchburyi*

Journal:	<i>Canadian Journal of Fisheries and Aquatic Sciences</i>
Manuscript ID	cjfas-2015-0401.R1
Manuscript Type:	Article
Date Submitted by the Author:	01-Nov-2015
Complete List of Authors:	O'Connell-Milne, Sorrel; University of Otago, Department of Marine Science Savage, Candida; University of Cape Town, Department of Biological Sciences Rayment, William; University of Otago, Department of Marine Science
Keyword:	Commercial harvesting, PARASITES < Organisms, Trematode, <i>Austrovenus stutchburyi</i> , Clam

The influence of commercial harvesting on parasite infection in the bivalve *Austrovenus stutchburyi*

Sorrel O'Connell-Milne¹

sorrel.a.milne@gmail.com

Candida Savage^{1,2}

candida.savage@otago.ac.nz

William Rayment¹

will.rayment@otago.ac.nz

¹Department of Marine Science, University of Otago, PO Box 56, Dunedin, New Zealand

²Department of Biological Sciences, University of Cape Town, Cape Town, South Africa

¹ Author for correspondence: sorrel.a.milne@gmail.com Tel: +64 27 7282913

Abstract:

Commercial harvesting often reduces densities of target species, potentially affecting transmission of parasites within the population. Rates of parasitic infection not only impact the hosts directly, but can result in wider ecosystem effects through influences on host behaviour. Commercial harvesting may therefore have important ecological consequences beyond the direct effects of removal of biomass. Clams (*Austrovenus stutchburyi*) are commonly infected by trematode parasites (*Acanthoparyphium* spp. and *Curtuteria australis*) and have been commercially harvested in Otago, New Zealand, since 1983. To assess the effects of harvesting on the abundance of trematodes in *A. stutchburyi*, the number of parasites per clam was compared in harvested and unharvested areas. The mean number of parasites per clam was 36% higher at commercially harvested sites, suggesting that harvesting enhances the local transmission of parasites. Harvesters may be able to reduce their influence on parasite infection levels in clam populations by harvesting less intensively and allowing more time between harvests for clam biomass to regenerate.

Key words: Commercial harvesting; Parasites; Trematode; *Austrovenus stutchburyi*; Clam

1. Introduction

Commercial harvesting reduces the population density of target species (Ludwig et al. 1993; Botsford et al. 1997), which can affect parasite transmission (Wood et al. 2010) and the intensity of infection experienced by individual hosts (Arneberg et al. 1998). When parasites are directly transmitted between individuals within a species, the reduced density of hosts may lower the transmission frequency of those parasites (Wood et al. 2010). However, as interactions between hosts (e.g. social interactions) can facilitate transmission, in some cases the parasite infection levels may persist and directly transmitted parasites may therefore be resilient to fishing pressure on their host (Wood & Lafferty 2014). Conversely, parasites with complex life cycles require multiple host taxa and may be more sensitive to the effects of harvesting, as the reduction in density of one of the host taxa may cause a 'life cycle bottleneck' reducing the efficiency of parasite transmission (Wood et al. 2010). When the parasites' definitive host is fished, the number of parasites within the system may be reduced as they are unable to complete their full life cycle (Wood & Lafferty 2014). Changes in parasite infection rates may affect the mortality of hosts directly (by killing the host) or indirectly (by changing the host's behaviour and facilitating predation), thereby altering transmission rates (Lafferty 2004, 2008; Sonnenholzner et al. 2011) or causing ecosystem shifts (Mouritsen & Poulin 2002). In the present study, the venerid bivalve *Austrovenus stutchburyi* was used to test whether harvesting affects parasite infection in clams.

The clam *A. stutchburyi* is an important target species for commercial, customary and recreational fisheries in New Zealand. Since the arrival of Māori in New Zealand, there has been a subsistence harvest of *A. stutchburyi* (Waselkov 1987). In Otago (south-eastern New Zealand), there is a combined recreational and customary harvest estimated at 20 tonnes per year (Ministry of Fisheries 2014) and a current commercial harvest of 800 tonnes per year out

of a total allowable commercial catch of 1,470 tonnes (Ministry of Fisheries 2014). The commercial fishery is operated by Southern Clams Ltd using a body dredge to manually harvest areas 60 cm wide by 2-3 m long. It is an intensive harvest selecting for shellfish greater than 27 mm and leading to an average reduction in biomass and density of the original stock by 70% and 55%, respectively (Irwin 2004). Although the reduction in density due to commercial harvesting has been assessed (Irwin 2004, Kainamu 2010), the effect of this change in host density on parasite infection has not been investigated.

The infective stages of echinostome trematodes *Acanthoparyphium* spp. and *Curtuteria australis* are acquired by the clam *A. stutchburyi* from the water column through the inhalant water current. These parasites then encyst in the foot of the clam as metacercariae (the juvenile parasite stage) (Martin & Adams 1961; Allison 1979), where they remain inactive in a state of hypobiosis (Chappell 1993). With a heavy parasite infestation, the encysted metacercariae reduce the clam's ability to use its foot to bury into the sediment (Thomas & Poulin 1998; Jensen et al. 1999; Mouritsen 2002). Individual clams stranded at the surface incur a seven-fold increase in susceptibility to predation by birds (Thomas & Poulin 1998). Therefore, while in the clam, the echinostomes positively influence their transmission from the clam to their definitive host, the South Island pied oystercatcher (*Haematopus ostralegus*) (Allison 1979). However, it also puts the parasite at risk of not reaching the terminal host, because fish such as the spotty (*Notolabrus celidotus*) may crop the tip from the foot of the surfaced clam along with any parasites encysted there (Mouritsen & Poulin 2003b).

Although the ecologically equivalent echinostome parasites *Acanthoparyphium* spp. and *C. australis* have been studied extensively, their distribution has not been investigated in the context of an anthropogenically altered ecosystem. Assuming cercariae (the parasite's

infective stage) actively search for their host and are approximately evenly distributed within the intertidal zone due to tidal water movement (de Montaudouin & Lanceleur 2011), high densities of clams at low-shore sites may indirectly ‘compete’ for parasites and clear the water of a finite number of cercariae (Mouritsen et al. 2003). Conversely, parasites may not become depleted from the water column at high-shore sites, where clams are at lower densities (Dobbinson et al. 1989; Mouritsen et al. 2003). The idea of parasites becoming ‘diluted’ in areas with high clam density by sharing the number of cercariae in the environment among more potential hosts has been proposed by Leung & Poulin (2007). This study will test that theory using areas with different clam densities resulting from harvesting pressure.

1.1 Objectives

The aim of the current study is to examine spatial variability of parasite accumulation within the clam *A. stutchburyi* in commercially harvested and unharvested areas. Field sampling provided a ‘snapshot’ which detailed the spatial fluctuations of parasite infection. Our hypothesis was that clams in harvested areas would have higher numbers of echinostome parasite infections due to a reduced density of hosts. Parasite infection levels in clams from commercially harvested and unharvested areas were compared while accounting for potentially confounding factors, such as clam age and body condition.

2 Methods

2.1 Study site

The study was conducted in November 2014 in three locations in Otago on the south-eastern coast of the South Island, New Zealand: inner Otago Harbour, outer Otago Harbour and

Blueskin Bay (Figure 1). These inlets support customary and recreational harvesting of *A. stutchburyi* and have designated commercial harvest areas.

Blueskin Bay (45°73'S, 170°59'E) was selected as the population of clams in this inlet has been commercially harvested for approximately 30 years, and detailed records exist of the intensity of harvest (Wildish 1984; Jiang et al. 2011). Blueskin Bay is a shallow bar-built inlet of 5.84 km² with extensive sand flats at low tide.

Otago Harbour (45°50'S, 170°35'E) is a long (23 km), narrow (mean width = 2 km), and typically shallow (mean depth = 4.5 m) tidal inlet covering 46 km². Two study locations were chosen in Otago Harbour; the inner harbour and the outer harbour. Intensive commercial harvesting of *A. stutchburyi* has taken place in Otago Harbour over the past three years (Ministry of Fisheries 2014).

2.2 Site selection and environmental characteristics

Paired harvested and unharvested areas with similar environmental conditions were selected at each of the three study locations. Candidate harvest sites were required to have had at least two commercial harvests within the last three years (data provided by Southern Clams Ltd), and an available 'control' site in an adjacent unharvested area. The term 'unharvested' is used to describe an area that has not been subjected to commercial harvesting. Recreational or customary harvesting may have taken place, however any shellfish removal was assumed to be negligible as the study sites were only accessible by boat. Candidate control sites were located as close as possible to the harvested sites, with a minimum distance of 15 m as a buffer zone from a harvested area.

To reduce the confounding effects of environmental gradients, potential study sites were required to be within 0.5 m of mean low water spring (MLWS) tide on the intertidal sand flats, resulting in similar immersion times. This ensured clams at all sites had a comparable time to feed and acquire parasites. Additionally, larger individuals and higher densities of clams at these low shore positions have been hypothesised to cause intraspecific competition for parasites (Mouritsen et al. 2003). Consequently, at this tidal elevation the effect of reduced clam density due to commercial harvesting on parasite infection may be more pronounced.

Fourteen sites, each including a paired harvested and control area, were randomly selected from a suite of candidate locations; four sites in each of the inner and outer Otago Harbour and six sites in Blueskin Bay (Figure 1).

For each study site, their elevation on the sand bank was recorded using a Trimble TSC2 GPS. A theoretical submergence rate was calculated from tidal predictions in the N.Z. Tidal Almanac. All sites had less than three hours of air exposure during each low tide. To assess substrate homogeneity among sites, three sediment samples were taken from within 5 m of each site using a 50 ml syringe corer to a depth of 5 cm (Fredensborg et al. 2005). To characterise the sediment, grain size and organic carbon were analysed for each sample. The percentage of organic carbon in the sediment was determined using mass loss on ignition (Mook & Hoskin 1982). Samples were pre-treated with hydrochloric acid (HCl) to remove shell fragments (Ryba & Burgess 2002). Sediment was dried for 12 hours at 105°C and ashed for four hours at 550°C, weighing after each step to the nearest 0.001g. The percentage of organic carbon in each sample was derived from its organic carbon content using the Van Bemmelen factor of 1.724, which assumes organic matter contains approximately 58%

carbon (Van Bemmelen 1890). Sediment grain size was measured with a laser diffraction particle size analyser (Malvern Mastersizer 2000). Samples were prepared for analysis using 10% hydrogen peroxide to remove organic matter, and Calgon to disperse the sediment particles (Singer et al. 1988). Grain size was used as an indicator of current flow, as finer particles settle in areas with slower water movement.

2.3 Spatial variability of parasites

At each study site, four 0.25 m² quadrats were randomly sampled along a 10 m transect, parallel to the water line at MLWS. Each quadrat was excavated to a depth of 0.1 m using a trowel. Sediment was washed through a 2 mm mesh sieve to retain shellfish. The retained clams were transported to the laboratory.

Clams were held for 24 hours in flowing filtered seawater to allow them to clear sediment from their digestive tract. They were washed and exogenous material removed, dried with a paper towel and weighed to the nearest 0.1 g using a digital balance. Clam density was quantified by counting clams within each sample. To ensure that the subsamples were representative of the various sized clams at each site, clams from each quadrat were sorted from largest to smallest and laid in rows of 10 – 20 individuals of decreasing size. A clam was selected from each row until a subsample of 20 clams was reached. If a sample had fewer than 20 clams, the sample was used in its entirety.

For each subsample, the length of each clam shell was measured to the nearest 0.1 mm from umbo to shell edge using digital callipers. Clams were aged by counting the number of growth interruption lines, observed as dark rings on the shell. Ages were recorded as categories of year groups, from age one to age seven. Clams eight years old and above were

combined into one age group, as ageing clams older than this using visual methods is difficult due to shell erosion. Although it is unclear whether the number of interruption lines identified are directly related to the number of years (McKinnon 1996), the number of lines correlate strongly with age and hence are useful in the current comparative context (Mouritsen 2002).

To assess the number of parasites, the foot of each clam was removed, by cutting along the narrow bridge between the gonad and foot base as described in Leung & Poulin (2011), then mounted on a glass slide and flattened beneath a cover slip (Leung et al. 2010). Encysted metacercariae within the foot were counted under a dissection microscope. No distinction was made between metacercariae of the two different echinostome taxa, *C. australis* and *Acanthoparyphium* spp.. This was because molecular markers are required to distinguish between the two taxa, and it was not deemed to be necessary as they have similar effects on the clam (Babirat et al. 2004). Hereafter, the term 'echinostomes' is used to refer to both of these parasite taxa collectively.

The soft tissue of each clam was removed and, along with the foot, oven-dried at 60°C for 24 hours, then weighed to the nearest 0.001 g to determine the soft-tissue dry-weight (STDW). To reduce the effect of gonadal variation on body weight, shellfish had been collected within the same week and prior to the spawning period.

Body condition of each clam was calculated using a residual index (Jakob et al. 1996). The STDW was regressed on clam shell length after the data were transformed to reciprocals to meet the assumptions of regression. The residual distances of individual points from the regression line then served as an indicator of whether the individuals' body condition was above or below that predicted for its length (Cone 1989). The body condition of clams was

not compared between treatments, as there may have been confounding effects from the availability of food and any effect of parasite infection on the clam.

2.4 Statistical analyses

All statistical analyses were carried out using the software package R 3.1.2 (R Development Core Team 2014). Variations in site elevation and sediment organic matter content, between harvest and control sites, were analysed using Student's t-tests.

Generalised Linear Mixed Models (GLMM) were used to assess the relationship between the dependant variables clam density per quadrat and clam biomass (wet weight) per quadrat, and the independent fixed factor 'treatment', with two levels: harvested and unharvested. 'Site' was treated as a random effect in the model. Models were constructed with a Gaussian error distribution and "identity" link function using the lme4 package (Bates et al. 2014). Simulation-based model diagnostics were used to compare the predicted outcomes based on the model to the raw data for both the density and biomass of clams (Appendix 1 & 2). This was done to observe whether the models' error structure fitted the distribution of the data (Pan & Lin 2005).

A suite of GLMMs was then used to assess the relationship between the number of parasites per clam and the fixed effects: 'treatment', 'body condition' and 'age'. 'Treatment' was a categorical variable with two levels (harvested and unharvested), and 'age' was treated as a continuous variable. The continuous variable 'body condition' was rescaled to create a z-score (Becker et al. 1988). The study location, site and replicate quadrat were treated as nested random effects in the model. Sites were nested within the three locations (inner Otago Harbour, outer Otago Harbour, and Blueskin Bay) due to an order of magnitude variation in

parasite infection between locations. Due to the zero-inflation of the overdispersed count data, separate models were trialled with negative binomial and Poisson error structures. Simulation-based model diagnostics were used to determine that a negative binomial error structure with a 'logit' link function was the best model structure for the data (Appendix 3).

All models were constructed for comparison using a manual backwards stepwise selection process. Akaike's Information Criterion with a correction for finite sample sizes (AICc) was used to identify the model with the best compromise between fit and complexity; i.e. the lowest AICc value. A difference of two AICc points was considered necessary to define the best model (Anderson & Burnham 2002). For the best fitting model, the multiplicative value for each fixed effect was derived by taking the exponential of the estimate (McCullagh & Nelder 1989). The change in the value of the response due to each fixed effect could then be derived by applying the multiplicative value. Note that the estimate for the rescaled variable 'body condition' had to be first unscaled by dividing by the standard deviation of the fixed effect.

3 Results

3.1 Site characteristics

All sites were within 0.5 m of MLWS in an area with a 2.1 m tidal range. There was no statistically significant difference in shore elevation between harvested and control areas (Student's t-test, $t_{42} = 1.27$; $p = 0.21$). There was also no significant difference between organic carbon content of the sediment in harvested and unharvested areas (Student's t-test, $t_{42} = 0.92$; $p = 0.36$). Sediment grain size between sites was homogeneous with all sites predominantly (> 97%) sand (62.5 – 1000 μm).

3.2 Spatial variability of parasites in clams

Infected clams were found at all study locations. The percentage of infected individuals ranged from 85% in the inner Otago Harbour to 97% in the outer Otago Harbour and Blueskin Bay. Parasite infection levels at the inner Otago Harbour sites were the lowest, with a mean infection of 13.6 (S.E. = 0.8) metacercariae per clam, whereas mean parasite infection at the outer Otago Harbour and Blueskin Bay sites were 197.2 (S.E. = 7.9) and 140.9 (S.E. = 5.4) metacercariae per clam, respectively.

The average densities of shellfish in harvested and unharvested areas were 66.6 and 101.4 individuals per quadrat, respectively. The average biomass (wet weight) of clams in harvested and unharvested areas was 752.3 and 1535.6 g, respectively. The GLMMs revealed that clam density was, on average, 37% lower in harvested areas than in unharvested areas, and that biomass of clams was 52% lower in harvested areas (Table 1).

The GLMM with treatment, age and body condition as predictors of parasite infection levels per clam emerged as clearly the best model (i.e. model with lowest AICc value; Table 2). All fixed effects had significant influences ($p < 0.001$) on parasite infection (Table 3). Harvested areas had 36% more parasites per clam on average than unharvested control areas. In addition, on average the number of parasite infections per clam increased by 61% with each additional year of age, and by 2% per unit of body condition.

4 Discussion

Commercial harvesting of *A. stutchburyi* reduced the density and biomass of the clam population, and these harvested populations contained over a third more echinostome

parasites than comparable unharvested populations. In the current study, we have demonstrated that harvesting influences the number of parasite infections in clams. This may, in turn, affect host behaviour, the surrounding ecological community and the commercial value of the harvested product.

The numbers of echinostome parasites infecting the clam *A. stutchburyi* were 36% higher in harvested areas than in unharvested areas once age and body condition of clams were accounted for. In unharvested areas, clams occurred at higher densities, which creates an encounter-dilution effect, lowering the chance of parasite infection (Mooring & Hart 1992). Following commercial harvest, this encounter-dilution effect is reduced and clams no longer 'compete' for parasites. Therefore, the parasite infection rate of remaining clams is elevated.

Clams with a higher body condition had greater numbers of echinostome parasites. This relationship was similar to the findings of Mouritsen et al. (2003). A positive correlation between parasite infection and body condition has often been interpreted to suggest that the encysted parasites (which are in a semi-dormant state) impose no measurable physiological cost on the clam host. This correlation is thought to be because individuals with a better body condition have a greater filtration rate (Coughlan & Ansell 1964), allowing uptake of more cercariae from the water column (Riisgard 2001; Mouritsen et al. 2003). However, the body condition of those same individuals may be even greater if parasites were not present. It has been demonstrated in controlled laboratory experiments that increasing numbers of parasite infections result in reduced growth and body condition of clams (Wegeberg & Jensen 2003; de Montaudouin et al. 2012a; O'Connell-Milne 2015). Therefore, it may be assumed that parasitism, by reducing the body condition of clams, may affect the commercial quality of clams as meat quantity is of importance to harvesters.

Older clams also had greater numbers of parasite infections. The pattern of older and larger clams harbouring more metacercariae has commonly been recorded in field-collected samples (Allison 1979; Poulin et al. 2000; Leung et al. 2009). The greater number of metacercariae in larger clams may be correlated with their greater filtration rate, as well as with parasite accumulation over time (Coughlan & Ansell 1964). The correlation between the age and shell size of clams in this study supports previous findings of a positive relationship between the shell length and parasite infection of clams observed locally (Thomas & Poulin 1998), and in the northern hemisphere (de Montaudouin et al. 1998). However, note that a recent study has found that clams may have a size range beyond which they become more resilient to infection (de Montaudouin et al. 2012b).

The parasite infection of clams varied among study locations, with greater numbers of infections per individual in the outer Otago Harbour and Blueskin Bay than in the inner Otago Harbour. However, due to the small distance between treatments, the distribution of cercariae was assumed to be homogeneous between harvest and control areas in the present study (Mouritsen et al. 2003). Small unrecognized gradients such as hydrodynamic conditions could in principle affect the rate at which clams accumulate parasites (Mouritsen 2002). The abundance of gastropods as first intermediate hosts and definitive avian hosts (oystercatchers) should be quantified in future studies to discern potential drivers of small-scale differences in parasite infection (Studer et al. 2013). The macrofaunal community (e.g., the mudflat anemone, *Anthopleura aureoradiata*, and wedge shell, *Macomona liliana*) and other parasites (e.g., Gymnophallidae) may also affect echinostome accumulation within clams. Gymnophallid parasites also encyst in *A. stutchburyi* and have a positive association with echinostome species (Poulin et al. 2000; Leung & Poulin 2007). The mudflat anemone

predates on parasites as they enter the water column surrounding the clams' intake siphon, therefore reducing the availability of parasites for *A. stutchburyi* (Mouritsen & Poulin 2003a). The wedge shell may act as a 'parasite sink', accumulating potentially infective cercariae and reducing the number of parasites in the local system (Leung & Poulin 2008). In future studies, the distribution of organisms with potential effects on the number of parasite infections in clams should be quantified.

When clam biomass is reduced by a commercial harvest, there is a reduction in food available for predatory whelks (*Cominella glandiformis*) and oystercatchers (*Haematopus ostralegus*), and less habitat for anemones (*Anthopleura aureoradiata*) that reside on clam shells (Mouritsen & Poulin 2003a). The reduced density of *A. stutchburyi* also lowers nutrient fluxes and potentially affects ecosystem productivity (Sandwell et al. 2009). Thus, along with increased parasite infection there are also further ecosystem consequences of the reduced density and biomass of the target population.

If the parasite infection level of individual clams is increased in a harvested area, this may have detrimental effects on the surrounding ecosystem. Greater infections result in shellfish stranded at the sediment surface and unable to bury themselves (Thomas & Poulin 1998; Jensen et al. 1999; Mouritsen 2002), which can increase their susceptibility to predation (Thomas & Poulin 1998). As heavily parasitised clams are easier prey for oystercatchers (*Haematopus ostralegus*), increased parasite infection may result in greater consumption of clams from harvested areas (Norris 1999). This increased predation in harvested areas may further slow the recovery of an already reduced biomass of clams. In contrast, increased parasite numbers in clams may have some beneficial effects. Once highly parasitised clams are stranded at the surface, they increase habitat heterogeneity (Mouritsen & Poulin 2002)

and provide substrate for attachment or shelter for epifauna, which in turn can increase the density and diversity of benthic organisms (Thomas & Poulin 1998; Mouritsen & Poulin 2005). In addition to limiting vertical movement, echinostomes also reduce horizontal movement of surfaced clams (Mouritsen 2002). Since bioturbation of the surface sediment by clams depresses the density of co-occurring infauna (Whitlatch et al. 1997), the reduced movement of clams may result in increased abundance of infauna (Mouritsen & Poulin 2002) and an associated increase in predator numbers (Reise 1985). The resulting enhanced local biodiversity may increase the resilience of the ecosystem to certain changes such as local extinctions or invasions (Mouritsen & Poulin 2002).

In summary, we found that clams from harvested sites had higher parasite infection levels than clams from adjacent unharvested areas, after accounting for differences in body condition and age. Assuming the environment experiences constant production and release of cercariae from gastropod primary intermediate hosts, the reduced biomass of the clam (the second intermediate host) may result in increased parasite infection per capita among the remaining clams. These changes in parasite infection may affect the clams directly by reducing their foot functionality or indirectly through increased predation pressure. In addition, increased parasite infections may cause ecosystem changes such as altered abundance of associated infauna, increased risk of avian predation, and variations to hydrodynamic processes and therefore sediment makeup. Further mechanistic studies investigating density as a driver of parasite infection are necessary to fully understand the consequences of commercial harvesting. If density is indeed the driver of parasite infection, as hypothesised in this study, commercial harvesters should consider how these effects may impact the wild stock to ensure sustainability into the future. Commercial harvesters could

reduce these impacts by harvesting less intensively or by leaving longer periods between subsequent harvests to allow the biomass of clams to recover.

Acknowledgments: We gratefully acknowledge those who were instrumental in making this work possible at the University of Otago especially Professor Robert Poulin, Tim Jowett and the volunteers who helped with collection of field data: Dave Redshaw, Angus Robertson, Janice Heung, Fatima George, Chris Niebuhr, Jesse Watts, Fanny Maure, Mathieu Morin, Anne Besson, Steph de Hamel, Tom McTavish, Georgia Bell and Brenton Twist. This study was funded by a grant from Southern Clams Ltd to S. O'Connell-Milne and the University of Otago publishing bursary.

Draft

References

- Allison, F.R. (1979) Life cycle of *Curtuteria australis* n.sp. (Digenea: Echinostomatidae: Himasthlinae), intestinal parasite of the South Island pied oystercatcher. *New Zealand Journal of Zoology* 6:13–20
- Anderson, D.R., Burnham, K.P. (2002) Avoiding pitfalls when using information-theoretic methods. *The Journal of Wildlife Management* 66:912–918
- Arneberg, P., Skorping, A., Grenfell, B., Read, A.F. (1998) Host densities as determinants of abundance in parasite communities. *Proceedings: Biological Sciences* 265:1283–1289
- Babirat, C., Mouritsen, K.N., Poulin, R. (2004) Equal partnership: two trematode species, not one, manipulate the burrowing behaviour of the New Zealand cockle, *Austrovenus stutchburyi*. *Journal of Helminthology* 78:195–199
- Bates, D., Maechler, M., Bolker, B., Walker, S. (2014) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* arXiv:1406.5823
- Becker, R.A., Chambers, J.M., Wilks, A.R. (1988) The New S Language. Wadsworth & Brooks/Cole, Pacific Grove
- Botsford, L.W., Castilla, J.C., Peterson, C.H. (1997) The management of fisheries and marine ecosystems. *Science* 277:509 – 515
- Chappell, L.H. (1993) Physiology and nutrition. In *Modern parasitology - a textbook of parasitology*. Edited by Cox F.E.G. Blackwell Science, Oxford, p 102 – 192
- Cone, S. (1989) The need to reconsider the use of condition indices in fishery science. *Transactions of the American Fisheries Society* 118:510 – 514
- Coughlan, J., Ansell, A.D. (1964) A direct method for determining the pumping rate of siphonate bivalves. *ICES Journal of Marine Science* 29:205–213

- de Montaudouin, X., Bazairi, H., Culloty, S. (2012a) Effect of trematode parasites on cockle *Cerastoderma edule* growth and condition index: A transplant experiment. *Marine Ecology Progress Series* 471:111–121
- de Montaudouin, X., Binias, C., Lassalle, G. (2012b) Assessing parasite community structure in cockles *Cerastoderma edule* at various spatio-temporal scales. *Estuarine, Coastal and Shelf Science* 110:54–60
- de Montaudouin, X., Wegeberg, A.M., Jensen, K.T., Sauriau, P.G. (1998) Infection characteristics of *Himasthla elongata* cercariae in cockles as a function of water current. *Diseases of Aquatic Organisms* 34:63–70
- Dobbinson, S.J., Barker, M.F., Jillett, J.B. (1989) Experimental shore level transplantation of the New Zealand cockle *Chione stutchburyi*. *Journal of Shellfish Research* 8:197 – 212
- Fredensborg, B.L., Mouritsen, K.N., Poulin, R. (2005) Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. *Marine Ecology Progress Series* 290:109–117
- Irwin, C. (2004) The impacts of harvesting and the sustainability of a New Zealand littleneck clam (*Austrovenus stutchburyi*) fishery in Papanui and Waitati Inlets, New Zealand. Ph.D. thesis, University of Otago
- Jakob, E.M., Marshall, S.D., Uetz, G.W. (1996) Estimating fitness: a comparison of body condition indices. *Oikos* 77:61–67
- Jensen, K.T., Castro, N.F., Bachelet, G. (1999) Infectivity of *Himasthla* spp. (Trematoda) in cockle (*Cerastoderma edule*) spat. *Journal of the Marine Biological Association* 79:265–271
- Jiang, W., Goodwin, E., Stewart, B. (2011) Stock assessment of clams (*Austrovenus stutchburyi*) in Waitati and Papanui Inlets, Otago, 2010 - 2011.

- Kainamu, A.A. (2010) The fishery trend and feeding capacity of the New Zealand Littleneck Clam, *Austrovenus stutchburyi*, in a southern New Zealand inlet. M.Sc. thesis, University of Otago
- Lafferty, K.D. (2004) Fishing for lobsters indirectly increases epidemics in sea urchins. *Ecological Applications* 14:1566–1573
- Lafferty, K.D. (2008) Ecosystem consequences of fish parasites. *Journal of Fish Biology* 73:2083–2093
- Leung, T.L.F., Keeney, D.B., Poulin, R. (2010) Genetics, intensity-dependence, and host manipulation in the trematode *Curtuteria australis*: following the strategies of others? *Oikos* 119:393–400
- Leung, T.L.F., Poulin, R. (2007) Recruitment rate of gymnophallid metacercariae in the New Zealand cockle *Austrovenus stutchburyi*: an experimental test of the hitch-hiking hypothesis. *Parasitology Research* 101:281–287
- Leung, T.L.F., Poulin, R. (2008) Size-dependent pattern of metacercariae accumulation in *Macomona liliana*: the threshold for infection in a dead-end host. *Parasitology Research* 104:177–180
- Leung, T.L.F., Poulin, R. (2011) Intra-host competition between co-infecting digeneans within a bivalve second intermediate host: dominance by priority-effect or taking advantage of others? *International Journal for Parasitology* 41:449–54
- Leung, T.L.F., Poulin, R., Keeney, D.B. (2009) Accumulation of diverse parasite genotypes within the bivalve second intermediate host of the digenean *Gymnophallus* sp. *International Journal for Parasitology* 39:327–31
- Ludwig, D., Hilborn, R., Walters, C. (1993) Uncertainty, resource exploitation, and conservation: lessons from history. *Science* 260:17–36

- Martin, W.E., Adams, J.E. (1961) Life cycle of *Acanthoparyphium spinulosum* Johnston, 1917 (Echinostomatidae: Trematoda). *The Journal of Parasitology* 47:777–782
- McCullagh, P., Nelder, J.A. (1989) Generalized linear models. Chapman & Hall, London
- McKinnon, J. (1996) Studies of the age, growth and shell increment patterns in the New Zealand cockle (*Austrovenus stutchburyi*). M.Sc. thesis, University of Otago
- Ministry of Fisheries (2014) Cockles (COC3) Otago Peninsula. Ministry of Fisheries, Wellington
- Mook, D.H., Hoskin, C.M. (1982) Organic determinations by ignition: caution advised. *Estuarine, Coastal and Shelf Science* 15:697–699
- Mooring, M.S., Hart, B.L. (1992) Animal grouping for protection from parasites: selfish herd and encounter-dilution effects. *Behaviour* 123:173–193
- Mouritsen, K.N. (2002) The parasite-induced surfacing behaviour in the cockle *Austrovenus stutchburyi*: a test of an alternative hypothesis and identification of potential mechanisms. *Parasitology* 124:521–528
- Mouritsen, K.N., McKechnie, S., Meenken, E., Toynbee, J.L., Poulin, R. (2003) Spatial heterogeneity in parasite loads in the New Zealand cockle: the importance of host condition and density. *Journal of the Marine Biological Association of the UK* 83:307–310
- Mouritsen, K.N., Poulin, R. (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* 124:S101–S117
- Mouritsen, K.N., Poulin, R. (2003a) The mud flat anemone-cockle association: mutualism in the intertidal zone? *Oecologia* 135:131–137
- Mouritsen, K.N., Poulin, R. (2003b) The risk of being at the top: foot-cropping in the New Zealand cockle *Austrovenus stutchburyi*. *Journal of the Marine Biological Association of the UK* 83:497–498

- Mouritsen, K.N., Poulin, R. (2005) Parasites boosts biodiversity and changes animal community structure by trait-mediated indirect effects. *Oikos* 108:340–350
- Norris, K. (1999) A trade-off between energy intake and exposure to parasites in oystercatchers feeding on a bivalve mollusc. *Proceedings: Biological Sciences* 266:1703–1709
- O’Connell-Milne, S.A. (2015) The effect of parasite load on clam (*Austrovenus stutchburyi*) growth rate and body condition in an environment modified by commercial harvesting. M.Sc. thesis, University of Otago
- Pan, Z., Lin, D.Y. (2005) Goodness-of-Fit methods for Generalized Linear Mixed Models. *Biometrics* 61:1000–1009
- Poulin, R., Steeper, M.J., Miller, A.A. (2000) Non-random patterns of host use by the different parasite species exploiting a cockle population. *Parasitology* 121:289–295
- R Development Core Team (2014) R: a language and environment for statistical computing.
- Reise, K. (1985) Tidal flat ecology: an experimental approach to species interactions. Springer-Verlag, Berlin
- Riisgard, H.U. (2001) On measurement of filtration rates in bivalves — the stony road to reliable data: review and interpretation. *Marine Ecology Progress Series* 211:275–291
- Ryba, S.A., Burgess, R.M. (2002) Effects of sample preparation on the measurement of organic carbon, hydrogen, nitrogen, sulfur, and oxygen concentrations in marine sediments. *Chemosphere* 48:139–147
- Sandwell, D.R., Pilditch, C.A., Lohrer, A.M. (2009) Density dependent effects of an infaunal suspension-feeding bivalve (*Austrovenus stutchburyi*) on sandflat nutrient fluxes and microphytobenthic productivity. *Journal of Experimental Marine Biology and Ecology* 373:16–25

- Singer, J.K., Anderson, J.B., Ledbetter, M.T., McCave, I.N., Jones, K.P.N., Wright, R. (1988)
An assessment of analytical techniques for the size analysis of fine-grained sediments.
Journal of Sedimentary Petrology 58:534–543
- Sonnenholzner, J., Lafferty, K.D., Ladah, L.B. (2011) Food webs and fishing affect
parasitism of the sea urchin *Eucidaris galapagensis* in the Galapagos. *Ecology* 92:2276–
2284
- Studer, A., Widmann, M., Poulin, R., Krkosek, M. (2013) Large scale patterns of trematode
parasitism in a bivalve host: no evidence for a latitudinal gradient in infection levels.
Marine Ecology Progress Series 491:125–135
- Thomas, F., Poulin, R. (1998) Manipulation of a mollusc by a trophically transmitted
parasite: convergent evolution or phylogenetic inheritance? *Parasitology* 116:431–436
- Van Bemmelen, J.M. (1890) Über die Bestimmung des Wassers, des Humus, des Schwefels,
der in den colloïdalen Silikaten gebundenen Kieselsäure, des Mangans u.s.w. im
Ackerboden. *Die Landwirthschaftlichen Versuchs-Stationen* 37:279–290
- Waselkov, G.A. (1987) Shellfish gathering and shell midden archaeology. *Advances in
Archaeological Method and Theory* 10:93–210
- Wegeberg, A.M., Jensen, K.T. (2003) In situ growth of juvenile cockles, *Cerastoderma
edule*, experimentally infected with larval trematodes (*Himasthla interrupta*). *Journal of
Sea Research* 50:37–43
- Whitlatch, R.B., Hines, A.H., Thrush, S.F., Hewitt, J.E., Cummings, V. (1997) Benthic faunal
responses to variations in patch density and patch size of a suspension-feeding
bivalve. *Journal of Experimental Marine Biology and Ecology* 216:171–189
- Wildish, K. (1984) An initial study of the New Zealand cockle (*Austrovenus stutchburyi*)
resource in Papanui and Waitati inlets. Report to the Ministry of Agriculture and
Fisheries

Wood, C.L., Lafferty, K.D. (2014) How have fisheries affected parasite communities?

Parasitology 142:134 – 144

Wood, C.L., Lafferty, K.D., Micheli, F. (2010) Fishing out marine parasites? Impacts of

fishing on rates of parasitism in the ocean. *Ecology Letters* 13:761–775

Draft

Table 1 Results of the GLMM investigating significance of the effect of harvesting on clam density and biomass. The multiplicative value for treatment is referenced against a control treatment.

Fixed effects	Estimate	Multiplicative value	Std. Error	t-value	Pr (> z)
<u>Density</u>					
(Intercept)	4.53	92.75856	0.1303	34.76	< 2e-16
Treatment	-0.4525	0.636036	0.059	-7.67	1.72E-14
<u>Biomass</u>					
(Intercept)	7.286117	1459.891	0.086321	84.41	< 2e-16
Treatment	-0.71399	0.489687	0.005949	-120.02	< 2e-16

Table 2 Candidate models for metacercarial infection in clams. Akaike's information criterion (AICc) and Δ AICc values of the models investigated, with parasite infection as the response variable (generalised linear mixed effect model with negative binomial error structures). Predictor variables are included in brackets. The model with the lowest AICc is in bold.

Candidate models	AICc	Δ AICc
Model 1 (Treatment, Age, Condition)	21903.8	0
Model 2 (Age, Condition)	21921.8	18
Model 4 (Treatment, Age)	21963.1	59.3
Model 3 (Age)	21981.4	77.6
Model 5 (Treatment, Condition)	23754.8	1851
Model 6 (Condition)	23755.2	1851.4
Model 7 (Treatment)	24546.8	2643

Table 3 Results of the GLMM investigating significance of the (fixed) effects age, condition and treatment on parasite infection of clams. The multiplicative value for treatment is referenced against a control treatment.

Fixed effects	Estimate	Multiplicative value	Std. Error	t-value	Pr (> z)
(Intercept)	0.735625	2.086786	0.649261	1.13	0.257
Age	0.477324	1.611756	0.008413	56.74	< 2e-16
Condition	0.01641	1.016546	0.027316	5.57	2.59E-08
Treatment	0.305278	1.357002	0.064805	4.71	2.47E-06

Draft

Figure 1 Location of the study area, and the paired harvested and unharvested study sites: 1 - 4 in the inner Otago Harbour, 5 - 8 in the outer Otago Harbour and 9 - 14 in Blueskin Bay.

Draft

Figure 1 Location of the study area, and the paired harvested and unharvested study sites: 1 - 4 in the inner Otago Harbour, 5 - 8 in the outer Otago Harbour and 9 - 14 in Blueskin Bay.

Appendix 1 Simulation-based model diagnostics plot for density with a Gaussian error structure and fixed factor treatment. Observed and simulated frequencies of densities of clams with groupings based on harvested (H) and unharvested (C) areas. The similar distribution of observed and simulated values shows the model is a good representation of this data set.

Appendix 2 Simulation-based model diagnostics plot for biomass with a Gaussian error structure and fixed factor treatment. Observed and simulated frequencies of biomass of clams with groupings based on harvested (H) and unharvested (C) areas. The similar distribution of observed and simulated values shows the model is a good representation of this data set.

Appendix 3 Simulation-based model diagnostics plot for snapshot field sampling model with negative binomial error structure and the fixed factors treatment, age and body condition. Observed and simulated frequencies of parasite infection averaged for age and treatment were plotted in four plots based on the clam's condition index. Plot 1 represents the quantile of clams with the lowest condition index, plot 2 and 3 the second and third quantiles, while plot 4 shows the quantile of clams with the highest condition index. The similar distribution of observed and simulated values shows the model is a good representation of this data set.