The roles of indirect interactions in a marine ecosystem

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CONTENTS

CHAPTER 1

General Introduction

Ecosystems are highly complicated systems in which organisms interact both directly and indirectly (Schmitz 2010). Understanding roles of indirect interactions in organizing ecosystems is one of recent challenges for ecologists. Early theories in community ecology (e.g. in intertidal rocky shores: Paine 1966, Fletcher 1987, Wootton 1992) assume that trophic cascades are initiated only when predators directly consume prey (consumptive effects, or CEs; Lima 1998). The reduced prey density transmits the effects to basal resources and hence indirect interactions were considered to be purely density-mediated (density-mediated indirect interactions, or DMIIs; Abrams 1995). However, in the 1990s, it is revealed that predators also modify behavioral, morphological, physiological, and life history traits of the prey (non-consumptive effects, or NCEs; Lima 1998). This leads to another form of indirect interactions, mediated by the alteration of traits of prey (trait-mediated indirect interactions, or TMIIs; Abrams 1995). Because changes in the species traits can have multiple consequences for other species directly and indirectly, NCEs and TMIIs have been recognized as important factors influencing community dynamics (Ohgushi et al. 2012).

Recent studies have advanced our understanding on the direct effects and indirect interactions. Many of these studies suggest that the strengths of NCEs and TMIIs may be similar or even greater than those of CEs and DMIIs (Peacor and Werner

2001, Trussell et al. 2006, 2008, Schmitz 2010, Matassa and Trussell 2011, 2014). Moreover, several factors have been identified to determine the strengths of DMIIs and TMIIs. They include foraging mode of predators (Henry 2010, Schmitz 2010), prey's hunger level (Matassa and Trussell 2014), and quality or quantity of resources (Luttbeg et al. 2003, Trussell et al. 2008).

However, there are four critical problems in the previous studies. First, the strengths of DMIIs and TMIIs have rarely been evaluated *in situ* and most experiments were conducted in the laboratory or controlled outdoor enclosures. Because chemicals from feeding predators are restricted to a small area, NCEs and TMIIs may be stronger in these experimental systems than expected in nature. Second, although herbivores often affect not only amount of plants but also their community structure in marine and terrestrial ecosystems (e.g. Trussell et al. 2004, Schmitz 2010), most studies of indirect interactions quantify only the amount, and the community structure is seldom studied (but see Trussell et al. 2004). Third, while characteristics of predators and resources in affecting such indirect interactions are well documented, relatively little attention has been paid to prey's characteristics. This situation is rather strange because it is prey that transmits top-down effects of their predators to resources. In particular, prey's density may be important because abundance of prey is directly related to the frequencies of interactions between predator and prey individuals, and between prey and resource individuals. Fourth, indirect interactions have been largely investigated in relatively short-term experiments (from days to weeks). This approach has limited our understanding on long-term dynamics of communities, especially because each component of ecosystems has seasonality and life history. Hence, it is necessary to evaluate the strengths of direct effects and indirect interactions in a long-term field experiment.

I conducted three field experiments in a marine food chain involving the carnivore snail *Thais clavigera*, its prey limpet *Siphonaria sirius*, and the prey's food algae *Lithoderma* sp. and *Ulva* sp. Because rocky shores are rather stable habitats and easy to access, they are suitable for continuous observations in comparison with other aquatic systems such as sea beds and rivers. In Chapter 2, I measured strengths of DMIIs and TMIIs, and how algal community changes, under natural predation pressure by *T. clavigera* on *S. sirius*. In Chapter 3, I experimentally manipulated the density of limpets to make low- and high-density plots, and measured strengths of DMIIs and TMIIs in each density condition. In Chapter 4, I conducted a 9-month field experiment involving seasonality of the components, and evaluated the fluctuating strengths of DMIIs and TMIIs.

LITERATURE CITED

Abrams, P. A. 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. The American Naturalist 146:112-134.

Fletcher, W. J. 1987. Interactions among subtidal Australian sea urchins, gastropods, and algae: effects of experimental removals. Ecological Monographs 57:89-109.

Henry, L. M., J. A. Bannerman, D. R. Gillespie, and B. D. Roitberg. 2010. Predator identity and the nature and strength of food web interactions. Journal of Animal Ecology 79:1164-1171.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions: What are the ecological effects of anti-predator decision-making? BioScience 48:25-34.

Luttbeg, B., L. Rowe, and M. Mangel. 2003. Prey state and experimental design affect relative size of trait- and density-mediated indirect effects. Ecology 84:1140-1150.

Matassa, C. M., and G. C. Trussell. 2011. Landscape of fear influences the relative importance of consumptive and nonconsumptive predator effects. Ecology 92:2258-2266.

Matassa, C. M., and G. C. Trussell. 2014. Prey state shapes the effects of temporal variation in predation risk. Proceedings of the Royal Society B 281:20141952.

Ohgushi, T., O. J. Schmitz, and R. D. Holt. 2012. Trait-mediated indirect interactions. Cambridge University Press, Cambridge.

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

Peacor, S. D., and E. E. Werner. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. Proceedings of the National Academy of Sciences of the United States of America 98:3904-3908.

Schmitz, O. J. 2010. Resolving ecosystem complexity. Princeton University Press, Princeton, New Jersey, USA.

Trussell, G. C., P. J. Ewanchuk, and C. M. Matassa. 2006. Habitat effects on the relative importance of trait- and density-mediated indirect interactions. Ecology Letters 9:1245-1252.

Trussell, G. C., P. J. Ewanchuk, and C. M. Matassa.2008. Resource identity modifies the influence of predation risk on ecosystem function. Ecology 89:2798-2807.

Trussell, G. C., P. J. Ewanchuk, M. D. Bertness, and B. R. Silliman. 2004. Trophic cascades in rocky shore tide pools: distinguishing lethal and nonlethal effects. Oecologia 139:427-432.

Wootton, J. T. 1992. Indirect effects, prey susceptibility, and habitat selection: impacts of birds on limpets and algae. Ecology 73:981-991.

CHAPTER 2

Changes in Algal Community Structure via Density- and Trait-mediated Indirect Interactions in a Marine Ecosystem

Abstract. In various terrestrial and aquatic ecosystems, predators affect resources indirectly via intermediate prey. Such indirect interactions involve reducing the density of the prey (density-mediated indirect interactions, DMIIs) or changing the behavioral, morphological or life history traits of the prey (trait-mediated indirect interactions, TMIIs). Although the importance of TMIIs has been highlighted recently, the strengths of both DMIIs and TMIIs under natural conditions have rarely been evaluated, especially in the context of resource community structure. I studied a three-level marine food chain involving the carnivorous snail *Thais clavigera*, its limpet prey *Siphonaria sirius* and the limpet's food sources, the algae *Lithoderma* sp. and *Ulva* sp. I measured the strengths of DMIIs and TMIIs and observed how the algal community changes under the pressure of natural predation by *T. clavigera* on *S. sirius*. Neither DMIIs nor TMIIs affected the total algal cover or chlorophyll content per unit area. However, both types of indirect interactions caused similar changes in algal composition by increasing the cover of *Ulva* and decreasing the cover of *Lithoderma*. This change in the algal community was caused by a reduction in the limpet's preferential consumption of the competitively dominant *Ulva* over *Lithoderma*. These results suggest that both DMIIs and TMIIs have similar effects on the changes in resource community structure under natural conditions.

INTRODUCTION

In various terrestrial and aquatic ecosystems, predators affect resources indirectly by reducing the prey's density (density-mediated indirect interactions, DMIIs) or by altering the prey's behavioral, morphological or life history traits (trait-mediated indirect interactions, TMIIs) (Schmitz 2010). As the prey often recognize the predators chemically or visually, TMIIs can spread immediately over a broad range, and such effects may last throughout the prey's lifetime (Trussell et al. 2003, Schmitz et al. 2004). Thus, growing evidence suggests that the magnitudes of TMIIs may be similar or even greater than those of DMIIs (Peacor and Werner 2001, Trussell et al. 2006, 2008, Schmitz 2010, Matassa and Trussell 2011). However, most of these previous studies were conducted in the laboratory or in controlled outdoor enclosures where chemical stimuli from feeding predators are restricted to a small area or are stronger than expected in nature. It is probable that these conditions result in the overestimation of the strength of TMIIs. Efforts have been made to estimate the natural strengths of DMIIs and TMIIs in terrestrial ecosystems (Schmitz 2010), but few such studies have been conducted in marine ecosystems (Trussell et al. 2004).

Indirect interactions can play important roles in shaping the local community and its functions (Dungan 1986, Wootton 1992, 1994, Douglass et al. 2008, Trussell et al. 2008, Schmitz 2010, Reynolds and Sotka 2011). However, very little is known about changes produced in resource community structure by DMIIs and TMIIs, except that both DMIIs and TMIIs involving the predatory crab *Carcinus maenas* and the herbivorous snail *Littorina littorea* increased the amount of the green algae *Enteromorpha* sp. (currently classified as *Ulva*) and *Ulva* sp. (Trussell et al. 2004). Because herbivores often affect plant community structure and its functions in many marine ecosystems (Paine 1980, Cubit 1984, Dungan 1986, Wootton 1992, Trussell et al. 2004, Douglass et al. 2008, Altieri et al. 2009, Reynolds and Sotka 2011) as well as terrestrial ecosystems (Belovsky and Slade 2000, Schmitz 2010), the top-down control of plant community structure through both DMIIs and TMIIs is probable and merits detailed study.

The relative strengths of DMIIs and TMIIs vary among study systems. These relative strengths depend on the characteristics of the system, such as resource availability or type (Wojdak and Luttbeg 2005, Trussell et al. 2008, Mooney et al. 2010), habitat type of the prey (especially whether risky or refuge; Trussell et al. 2006, Schmitz 2010, Matassa and Trussell 2011), or the hunting mode of predators (Schmitz 2008, Henry et al. 2010). The strengths of DMIIs and TMIIs may also change over time as prey individuals can respond to the cue of danger immediately, whereas density reduction may affect neighboring individuals gradually. Although such possibility has been suggested in theoretical (Abrams 2008) or experimental (Hoverman and Relyea 2012) studies, the time-dependent nature of the strengths of DMIIs and TMIIs is not well understood in the field.

In addition, as in the case of DMIIs or TMIIs, predators affect the prey's characteristics directly via reduction of the prey density (consumptive effects) or via responses to the predator cues (non-consumptive effects). Because direct effects are the pathways of indirect interactions, it is important to study direct effects to understand the mechanisms and strengths of indirect interactions. However, it is not well understood how the relative strengths of DMIIs and TMIIs are determined by consumptive and non-consumptive direct effects on the prey.

I investigated the relative strengths of top-down DMIIs and TMIIs as well as consumptive and non-consumptive effects in a simple trophic cascade consisting of the carnivorous muricid snail *Thais clavigera*, the pulmonate limpet *Siphonaria sirius* (Pilsbry), and the green alga *Ulva* sp. and the cyanobacterium (blue-green “alga”) *Lithoderma* sp. (Fig. 1). The limpet, *S. sirius*, inhabits the lower intertidal zone of rocky shores and shows homing behavior after feeding excursions, although they are not territorial (Ohgushi et al. 1953). The rock surfaces within the home ranges of the limpets are covered with encrusting algae, such as *Lithoderma* sp. or the brown alga *Ralfsia* sp. These encrusting algae are maintained by the limpet’s preferential grazing on the foliose green algae *Ulva* sp., which are superior competitors (Iwasaki 1993a). Because *S. sirius* respond by fleeing when attacked by *T. clavigera* (Iwasaki 1993b), I hypothesized that both DMIIs and TMIIs affect not only the biomass but also the community structure of the algae. I assessed the strengths of the consumptive and non-consumptive effects of the predator on the limpet’s growth, survival and *per capita* feeding rates. I also studied the effects of the resultant DMIIs and TMIIs on algal community structure (the total coverage, percentage of *Ulva* in the algae and chlorophyll content per unit area) under the pressure of natural predation by the snail on the limpet, within one week and over more than one month.

MATERIALS AND METHODS

Experimental plots

I conducted a field experiment near Seto Marine Biological Laboratory, Wakayama, Japan (33.75° N, 135.28° E). I selected four sites in the lower intertidal area at slightly different tidal levels (from -51 cm to -30 cm relative to the mean tidal level). Each site was 15.9 – 29.4 m^2 and was adjacent to each other. In each site, I selected six sandstone rocks (24 in all) that were apart at least 0.9 m from one another. Thus, the limpets could not migrate between the rocks. The uppermost flat side of each rock was used as the experimental plot (each 0.18 – 0.72 m^2). The experiment lasted 38 days, from 15 August through 21 September 2010. During this season, *S. sirius* actively forage and reproduce (Iwasaki 1995a, b), but its larval recruitment does not occur (Iwasaki 1993c). The green alga *Ulva* sp. increases in cover from late summer toward winter in this area (Iwasaki 1993c). Censuses were made at intervals of 6 – 12 days.

The experiment included two treatments applied to the limpets in accordance with the standard experimental design (e.g., Peacor and Werner 2001, Griffin and Thaler 2006, Trussell et al. 2006, Yoshie and Yusa 2011). First, a “consumptive treatment” was used to simulate the direct consumption of the prey *S. sirius* by the predators. This procedure enabled us to estimate the strengths of the consumptive effects and resulting

DMIIs. I used a scheduled removal of the prey. This approach is customary because introducing natural predators will inevitably release odors and is unsuitable for distinguishing consumptive and non-consumptive effects (Peacor and Werner 2001, Griffin and Thaler 2006, Trussell et al. 2006). Second, a “non-consumptive treatment”, in which odors from caged feeding predators were released, was used to quantify the strengths of non-consumptive effects and TMIIs. These treatments were combined to make four treatment groups: “consumptive treatment only”, “non-consumptive treatment only”, both treatments (“consumptive and non-consumptive”), and neither treatment (“control”). *S. sirius* was the dominant herbivorous gastropod at the study site, and other herbivores as well as all carnivores were removed from these plots. I also established “no-limpet” plots. All *S. sirius* as well as other herbivores were removed from these plots. This treatment was applied to observe the change in the algal community in the absence of herbivores. Moreover, I made “natural” plots that maintained natural conditions (without consumptive or non-consumptive treatments and with no removal of animals) to estimate the natural predation rates on *S. sirius*. Thirty randomly selected individuals of *S. sirius* (8.0 – 24.3 mm in shell length) in each plot were individually marked with paint. A preliminary survey revealed that the paint had no apparent effects on the behavior and survival of the limpets. The initial shell lengths did not differ significantly among the five treatment groups (ANOVA; $F_{4, 15} = 0.29$, $P = 0.88$; except for no-limpet plots). The six treatment groups (consumptive, non-consumptive, consumptive and non-consumptive, control, no-limpet, and natural treatment) were randomly assigned to one of the six rocks at each site in the study area. In order to prevent animals from moving into or out of the plots, the edge of each experimental plot, except for the natural plot, was lined (~5 cm width) with paints containing copper powder (Denka, Tokyo, Japan) as in the experiments by Cubit (1984).

Experimental manipulations

As the consumptive treatment, I removed a percentage of both the marked and the unmarked limpets with a scraper at each census. This percentage (4－12%; average 8.0% per census) was determined based on the natural predation rate on limpets (Mnat －Mcont), where Mnat is the average mortality in natural plots (with predators) and Mcont is the average mortality in control plots (without predators) during the previous interval of the census. The first consumptive treatment at the start of the experiment was determined based on the natural mortality of 100 randomly selected individuals on a rock in the study area during the previous two weeks (8.2% per week).

To perform the non-consumptive treatment, I attached a mesh cage (25 mm high, 77 mm diameter) with water-resistant epoxy putty (Konishi Corporation, Osaka, Japan) to the upper center of each plot except for the natural and no-limpet plots. The top (made of stainless steel mesh) was screwed to the side (plastic) of the cage. The side of the cage was perforated to permit the release of odors of *T. clavigera* feeding on *S. sirius*. I put *T. clavigera* ("C type" *sensu* Abe [1985]; shell height 20.4 – 25.2 mm) and *S. sirius* (shell length 8.0 – 24.3 mm) in each cage of the plots with the non-consumptive treatment. The number of *S. sirius* used was based on the natural predation rate (i.e., equal to the proportion removed in the consumptive treatment). The number of *T. clavigera* used was equal to the average number of carnivorous snails (8 – 11 individuals) in the natural plot measured at each census. Both *T. clavigera* and another muricid, *Morula musiva*, were counted as carnivorous snails because the responses of *S. sirius* to *M. musiva* and to *T. clavigera* are similar (N. Abe, *unpublished data*). The survival of *T. clavigera* in the cage was checked occasionally, and dead individuals were replaced with new ones. No predators and limpets were put into the cages for the control plots and the plots with consumptive treatment only.

Additional experiments were conducted to examine the effects of the copper paint and setting cages with epoxy putty on survival, growth and activity of the limpets in the plots in 2012 (see Appendix: Table A1). The results indicated that the artificial treatments did not affect survival, growth or activity of the limpets. Moreover, very few limpets migrated across the boundary of the treatment plot in the absence as well as the presence of copper paint. Thus, each experimental plot was large enough for the limpets to complete all the activities including grazing, reproduction and escaping from predators.

Estimation of direct effects and indirect interactions

Whenever data were available, I analyzed the initial (one week after the start) and the final changes (at the end of the 38-day experiment) for both direct effects and indirect interactions. To estimate the consumptive and non-consumptive effects on *S. sirius*, I calculated the growth rate, the percentage of individuals that disappeared, and the *per capita* feeding rate on *Ulva* sp. The growth rate was expressed as the shell length at the final measurement (28 days after the start of the experiment) relative to the initial measurement. To calculate the percentage of individuals that disappeared, the number of marked individuals that disappeared was checked at each census, and this value was divided by the number of survivors at the previous census. This percentage was measured four times and averaged.

The *per capita* feeding rate of limpets on *Ulva* sp. in each treatment group (consumptive, non-consumptive, both treatments and control) was calculated as the mean percent cover of *Ulva* sp. in no-limpet plots minus the percent cover of *Ulva* sp. in each plot in the treatment group, divided by the cumulative number of limpets in the plot during the experiment. By using the mean value, statistical independence of data from each plot was secured. The cumulative number of limpets was calculated as the number of individuals that survived until each census × the days from the beginning of the experiment until the census (Yoshie and Yusa 2011). A dead individual was assumed to have died on the day midway between the time when it was last found alive and the time when it was first found dead or found to have disappeared. Thus, the *per capita* feeding rate reflects the average quantity of *Ulva* eaten per *S. sirius* individual per day in each plot. The *per capita* feeding rate on *Lithoderma* was not calculated because its cover decreased if limpets were eliminated.

To estimate the DMIIs and TMIIs of the predator on the algae, I measured the percent cover of each algal species and the amount of chlorophyll (amounts of chlorophyll *a* and *b*) per unit area of rock surface. To calculate the percent cover, I placed two quadrats (18.5×18.5 cm) just below the cage and on the right side, and then I photographed the quadrats. I plotted 169 equidistant dots on each quadrat on a personal computer and counted the number of points superimposed on each algal species. This procedure was repeated five times during the observation period and averaged. To estimate the amounts of chlorophyll *a*, chlorophyll *b* and chlorophyll *a* + *b*, I scraped off all algae within three 1×1 cm quadrats each at locations 5, 10, and 15 cm distant from the cage (the direction was determined systematically). I combined data from the three samples from each plot because no effect of the distance on chlorophyll *a* + *b* was found ($F_{2,69} = 1.45$, $P = 0.23$). After fixing the samples in 5 mL of a solution of 90% acetone: 10% pure water, I measured the chlorophyll by the trichrometric method (Saijo 1975). Because the sampling of the algae could be conducted only at spring low tides, the measurement was performed two weeks after the start and at the end of the experiment.

Statistical analyses

I analyzed the data with a general linear model in JMP version 9 (SAS Institute, Cary, North Carolina, USA). To test the effect of each treatment on the limpet and the algae, I included the presence/absence of consumptive and non-consumptive treatments and their interaction term as explanatory variables. Moreover, the mean tidal level of each site, as well as its two- or three-order interaction terms with consumptive and non-consumptive treatments, was also included in the model. The effects of tidal level was included as it is known to affect the mortality of a congener limpet, *Siphonaria* *diemenensis* Quoy et Gaimard (Quinn, 1988). All explanatory variables were treated as fixed factors. In this study, the data of limpets that were manually removed in the consumptive treatment were not included, because I was interested in the effects of removal (simulated predation) on the remaining limpets as direct effects (although the removed limpets were considered in interpreting the results of indirect interactions). Percentage data were arcsine transformed before analysis to meet the assumptions of the model.

RESULTS

Direct effects on limpets

The growth rate of *Siphonaria sirius* was reduced by the non-consumptive treatment (Fig. 2), whereas other effects including consumptive treatment, the mean tidal level, and their interaction terms were not significant (Table 1).

The percentage of *S. sirius* individuals that disappeared increased under the non-consumptive treatment after the first week (Appendix: Fig. A1a). Other effects were not significant (Table 1). Similarly, the non-consumptive treatment increased the percentage of limpets that disappeared over the entire period (Appendix: Fig. A1b). Moreover, the effects of the tidal level and the interaction term between tidal level and consumptive treatment were significant; the percentage of limpets that disappeared increased with increasing tidal levels (Table 1). The average percentage of individuals that disappeared per census increased by 8.8% by non-consumptive treatment over the entire period.

The *per capita* feeding rate on *Ulva* sp. by *S. sirius* was suppressed by the non-consumptive treatment in the first week (Appendix: Fig. A2a), whereas other effects were not significant (Table 1). None of these variables affected the feeding rate in the entire period (Appendix: Fig. A2b; Table 1).

Indirect interactions with algae

Most rock surfaces (ca. 90%) in the experimental plots were covered with algae for most of the experimental period (Fig. 3a). Among the algae, *Ulva* and *Lithoderma* were dominant, comprising >90% of the total algal cover. However, the temporal changes in the two algae were strikingly different. The percent cover of *Ulva* sp. increased with time in all treatment groups (Fig. 3b). The increase was most marked in the no-limpet plots, whereas the natural or control plots showed only a small increase. In contrast, *Lithoderma* sp. decreased with time. Thus, the coverage of *Lithoderma* showed a negative correlation with the cover of *Ulva* within each treatment group (e.g., in no-limpet plots, $r = -0.99$, $P < 0.001$, $N = 5$ censuses).

No effects of the treatments on the total cover of the algae were detected in the first week (Table 2). However, the percentage of algal coverage represented by *Ulva* was affected by the treatment within one week. TMIIs increased the percentage of *Ulva* in the total algal cover, whereas other effects were not significant (Table 2). At the end of the experiment, there were no significant effects of the treatments on the total cover of the algae, either (Fig. 4a; Table 2). On the other hand, both DMIIs and

TMIIs increased the percentage of *Ulva* (Fig. 4b), although other effects were not significant (Table 2). The effects of treatments on *Lithoderma* were not tested because of the lack of statistical independence due to the highly negative correlation between the cover of *Ulva* and that of *Lithoderma*.

The amounts of chlorophyll *a*, chlorophyll *b* and chlorophyll *a* + *b* per unit area were not influenced by the consumptive or non-consumptive treatment or their interaction term, either after two weeks or at the end of the experimental period (Appendix: Table A2).

DISCUSSION

I hypothesized that both DMIIs and TMIIs affected not only the amount of algae but also the community structure of the algae in a simple marine trophic cascade including a snail, a limpet and two groups of algae. I assessed the strengths of the DMIIs and TMIIs under natural predation pressure by the snail on the limpet. As mechanisms for the indirect interactions, I also assessed the strengths of consumptive and non-consumptive effects. Contrary to my hypothesis, I found that neither the DMIIs nor TMIIs affected the total algal cover. However, the indirect interactions affected the composition of the algae (Table 3), supporting my hypothesis. The effects of the TMIIs were more rapid than the effects of the DMIIs, but the strengths of the TMIIs and DMIIs in the final analysis were similar. The non-consumptive effects on the limpets' growth, the percentage of the limpets that disappeared, and the *per capita* feeding rates were stronger than the consumptive effects (Table 3). In the following, I discuss the possible mechanisms and implications of these findings.

Direct effects

In general, prey traits are controlled by trade-offs between predation risk and foraging (Lima and Dill 1990, Lima 1998, Trussell et al. 2006, Schmitz 2010). In the present case, limpets that sensed odors of feeding predators in the non-consumptive treatment reduced their *per capita* feeding rate. In turn, this response resulted in a reduced growth rate due to lower energy gain. In addition, the energy required to escape from predators may be partly responsible for the reduced growth, as *S. sirius* individuals crawl away when attacked by *T. clavigera* (Iwasaki 1993b). The greater percentage of individuals that disappeared suggests a higher mortality resulting from the reduced energy gain (i.e., starvation) and an increased energy loss required for escape behavior. In fact, many empty shells of marked *S. sirius* were found near the experimental plots. Additional experiments (Appendix: Table A1) indicated that few limpets escaped outside the experimental plots, suggesting that the greater percentage of individuals that disappeared in the non-consumptive treatment plots in the original experiment was due to higher mortality of limpets.

Two factors may explain the difference between the higher non-consumptive effects and the lower consumptive effects. First, as commonly invoked in other systems (Trussell et al. 2003, Schmitz et al. 2004), the odors from feeding predators in the non-consumptive treatment spread immediately over a broad range and might have affected many limpets. In contrast, the removal of limpets in the consumptive treatment would have affected only the neighboring individuals, as individual *S. sirius* maintain fixed home ranges in which they forage (Ohgushi et al. 1953) and individuals would not have expanded their home ranges soon after the removal of their neighbors.

Second, the natural mortality of *S. sirius* is lower than the mortalities of other limpets with no homing habit (Iwasaki 1993c). Because the number of *S. sirius* removed in the consumptive treatment was based on the estimated predation rates, this treatment resulted in lower mortality by simulated predation (i.e., manual removal) and, hence, in small consumptive effects. Low predation rates, however, should be regarded as a result of well-developed antipredator behavioral strategies rather than low predation pressure, as *S. sirius* has marked escape responses (Iwasaki 1993b), and other behavioral traits such as diurnal foraging and spawning rhythms are also considered to be affected by predation pressure (Iwasaki 1995a).

These observations highlight the importance of the prey’s life history parameters in determining the strengths of both consumptive and non-consumptive effects. Highly developed antipredator responses as well as predator-induced shifts in growth, foraging and possibly reproductive strategies will affect mortality due to predation and, hence, the strengths of both non-consumptive and consumptive effects. The mode of competition, including territoriality (e.g., Stimson 1970, 1973), may also be important in determining the strengths of consumptive effects. To date, several factors have been suggested as determinants of the strengths of consumptive and non-consumptive effects, including the quality and quantity of resources (Trussell et al. 2008, Preisser et al. 2009) and the foraging mode of predators (Rundle et al. 2003, Schmitz 2010). Although they are less well studied, prey traits such as the habitat domain (Schmitz 2010) and the mode of density dependence (Yoshie and Yusa 2011) have been also suggested. Because consumptive and non-consumptive effects may have profound influences on indirect interactions and the resulting community structure, the importance of such prey traits, including the prey's life history parameters, should be further explored.

A significant effect of the tidal level, and its interaction term with the consumptive treatment, was detected only for the percentage of *S.sirius* individuals that disappeared in the entire period. This suggests that the effects of tidal level on the other direct and indirect interactions were not so important in this study area. The percentage of individuals that disappeared increased with increasing tidal level. This is consistent with the observation that the mortality of the congener limpet *S. diemenensis* was higher in the upper tidal zone than in the lower zone (Quinn, 1988).

Indirect interactions

The more rapid transmission of TMIIs than DMIIs to the algal community is most likely related to the mechanisms involved. In this study, the mechanisms of TMIIs are summarized as follows: the odors of the feeding predators reduced the *per capita* feeding rate as well as the number of living limpets, and these reductions resulted in the rapid increase of the competitively dominant alga *Ulva* and the decrease of the subordinate *Lithoderma*. As no consumptive effects of predators on the limpets were detected in this study, the mechanism of the DMIIs was a density reduction due to the consumptive treatment (scheduled removal) itself. Because the removal was made gradually, its effects were also gradual. Because my removal schedule reflects the natural predation rates, the time dependence of the relative importance of the DMIIs and TMIIs should also be expected in nature. Although most studies on indirect interactions in marine communities have been based on short-term observations, I agree with Wootton (1992), Abrams (2008), and Hoverman and Relyea (2012) who stress the need to understand the time-dependent nature of indirect interactions, especially the relative strengths of DMIIs and TMIIs. The present experiment could detect such temporal changes in the strengths of DMIIs and TMIIs, although the experiment was not long enough to cover a whole life span of the limpets (presumably 2 - 3 years; Iwasaki 1993c). If I had continued the present experiment, the relative strength of DMIIs and TMIIs would have further changed due to seasonality in life history traits of the limpets such as larval recruitment in autumn and low activity in winter.

The final results showed that the percentage of *Ulva* increased both through DMIIs and TMIIs. Moreover, the magnitude of the effects was similar, i.e., both DMIIs and TMIIs produced similar changes in the algal community although the total algal coverage was unchanged. I removed the limpets in the consumptive treatments by 8.0% per census on average, and the effect of this removal was balanced with an increased percentage of limpet individuals that disappeared by the non-consumptive treatment (average 8.8% per census). The reduced *per capita* feeding rates of the remaining individuals by the non-consumptive treatment would have further increased the magnitude of TMIIs, although such an increase was not detected statistically. Because my treatments were based on natural predation rates, I expect that the DMIIs and TMIIs were of similar strengths in the study site on this time scale.

In summary, the non-consumptive effects by the predators on the limpet were stronger than the consumptive effects. Indirect interactions changed the algal community structure but not the total algal coverage. The TMIIs on the algae were stronger than the DMIIs within the first week, but they were of similar magnitudes over the entire experimental period. In addition to the characteristics of predators and resources, I stress the importance of prey characteristics, such as density-dependent responses and life history strategies, in shaping resource community structure.

LITERATURE CITED

Abe, N. 1985. Two forms of *Thais clavigera* (Küster, 1858). Venus 44:15–26.

Abrams, P. A. 2008. Measuring the impact of dynamic antipredator traits on predator-prey-resource interactions. Ecology 89:1640-1649.

Altieri, A. H., G. C. Trussell, P. J. Ewanchuk, G. Bernatchez, and M. E. S. Bracken. 2009. Consumers control diversity and functioning of a natural marine ecosystem. PLoS ONE 4 e5291:1-6.

Belovsky, G. E., and J. B. Slade 2000. Insect herbivory accelerates nutrient cycling and increases plant production. Proceedings of the National Academy of Sciences USA 97:14412-14417.

Cubit, J. D. 1984. Herbivory and the seasonal abundance of algae on high intertidal rocky shore. Ecology 65:1904-1917.

Douglass, J. G., J. E. Duffy, and J. F. Bruno. 2008. Herbivore and predator diversity interactively affect ecosystem properties in an experimental marine community. Ecology Letters 11:598-608.

Dungan, M. L. 1986. Three-way interactions: barnacles, limpets, and algae in a Sonoran Desert rocky intertidal zone. American Naturalist 127:292-316.

Griffin, C. A. M., and J. S. Thaler. 2006. Insect predators affect plant resistance via density- and trait-mediated indirect interactions. Ecology Letters 9:338-346.

Henry, L. M., J. A. Bannerman, D. R. Gillespie, and B. D. Roitberg. 2010. Predator identity and the nature and strength of food web interactions. Journal of Animal Ecology 79:1164-1171.

Hoverman, J. T., and R. A. Relyea. 2012. The long-term impacts of predators on prey: inducible defenses, population dynamics, and indirect effects. Oikos 121:1219-1230.

Iwasaki, K. 1993a. Synergistic effects of mixed grazing by intertidal limpets on sessile organisms: consequences of differences in grazing ability and feeding habit. Physiology and Ecology Japan 30:1-30.

Iwasaki, K. 1993b. Analyses of limpet defense and predator offense in the field. Marine Biology 116:277-289.

Iwasaki, K. 1993c. The role of individual variability in limpet resting site fidelity and competitive ability in the organization of a local rocky intertidal community. Physiology and Ecology Japan 30:31-70.

Iwasaki, K. 1995a. Foraging and spawning rhythms of the pulmonate limpet *Siphonaria sirius* (Pilsbry): switching of activity period by a diurnal forager. Journal of Molluscan Studies 61:275-288.

Iwasaki, K. 1995b. Dominance order and resting site fidelity in the intertidal pulmonate limpet *Siphonaria sirius* (Pilsbry). Ecological Research 10:105-115.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions: What are the ecological effects of anti-predator decision-making? BioScience 48:25-34.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68:619-640.

Matassa, C. M., and G. C. Trussell. 2011. Landscape of fear influences the relative importance of consumptive and nonconsumptive predator effects. Ecology 92:2258-2266.

Mooney, K. A., R. Halitschke, A. Kessler, and A. A. Agrawal. 2010. Evolutionary trade-offs in plants mediate the strength of trophic cascades. Science 327:1642-1644.

Ohgushi, R., T. Miura, and M. Kotani. 1953. Some observations on the homing behavior of two species of limpets. Venus (Japanese Journal of Malacology) 17:219-223.

Paine, R. T. 1980. Food webs: linkage, interaction strength and community infrastructure. Journal of Animal Ecology 49:667-685.

Peacor, S. D., and E. E. Werner. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. Proceedings of the National Academy of Sciences USA 98:3904-3908.

Preisser, E. L., D. I. Bolnick, and J. H. Grabowski. 2009. Resource dynamics influence the strength of non-consumptive predator effects on prey. Ecology Letters 12:315-323.

Quinn, G. P. 1988. Ecology of the intertidal pulmonate limpet *Siphonaria diemenensis* Quoy et Gaimard. I. Population dynamics and availability of food. Journal of Experimental Marine Biology and Ecology 117:115-136.

Reynolds, P. L., and E. E. Sotka. 2011. Non-consumptive predator effects indirectly influence marine plant biomass and palatability. Journal of Ecology 99:1272-1281.

Rundle, H. D., S. M. Vamosi, and D. Schluter. 2003. Experimental test of predation’s effect on divergent selection during character displacement in sticklebacks. Proceedings of the National Academy of Sciences USA 100:14943-14948.

Saijo, Y. 1975. Methods for measurements of chlorophyll. Japanese Journal of Limnology 36:103-109.

Schmitz, O. J. 2008. Effects of predator hunting mode on grassland ecosystem function. Science 319:952-954.

Schmitz, O. J. 2010. Resolving ecosystem complexity. Princeton University Press, Princeton, New Jersey, USA.

Schmitz, O. J., V. Krivan, and O. Ovadia. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. Ecology Letters 7:153-163.

Stimson, J. 1970. Territorial behavior of the owl limpet, *Lottia gigantea*. Ecology 51:113-118.

Stimson, J. 1973. The role of the territory in the ecology of the intertidal limpet *Lottia gigantea* (Gray). Ecology 54:1020-1030.

Trussell, G. C., P. J. Ewanchuk, and M. D. Bertness. 2003. Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. Ecology 84:629-640.

Trussell, G. C., P. J. Ewanchuk, M. D. Bertness, and B. R. Silliman. 2004. Trophic cascades in rocky shore tide pools: distinguishing lethal and nonlethal effects. Oecologia 139:427-432.

Trussell, G. C., P. J. Ewanchuk, and C. M. Matassa. 2006. Habitat effects on the relative importance of trait- and density-mediated indirect interactions. Ecology Letters 9:1245-1252.

Trussell, G. C., P. J. Ewanchuk, and C. M. Matassa. 2008. Resource identity modifies the influence of predation risk on ecosystem function. Ecology 89:2798-2807.

Wojdak, M. J., and B. Luttbeg. 2005. Relative strengths of trait-mediated and density-mediated indirect effects of a predator vary with resource levels in a freshwater food chain. Oikos 111:592-598.

Wootton, J. T. 1992. Indirect effects, prey susceptibility, and habitat selection: impacts of birds on limpets and algae. Ecology 73:981-991.

Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics 25:443-466.

Yoshie, H., and Y. Yusa. 2011. Indirect interactions in a rice ecosystem: density dependence and the interplay between consumptive and non-consumptive effects of predators. Freshwater Biology 56:302-310.

FIGURES

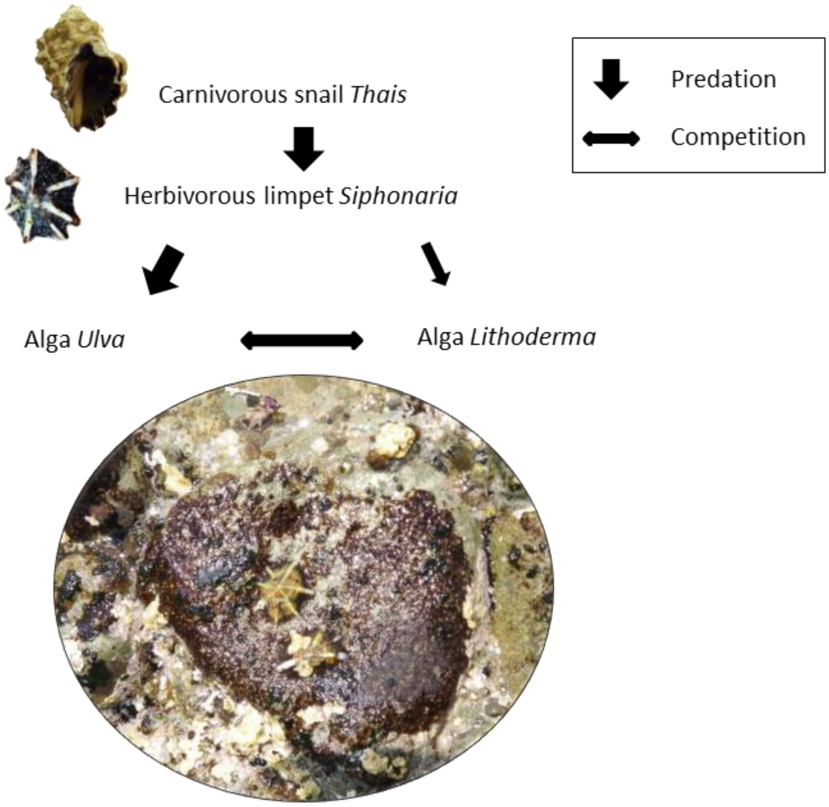

Fig. 1. A simple trophic cascade consisting of the carnivorous snail *Thais clavigera*, the limpet *Siphonaria sirius*, and the algae *Ulva* sp. and *Lithoderma* sp. *Lithoderma* is competitively inferior to *Ulva* but is maintained by the limpet's preferential feeding on *Ulva* within its home range. The snail is expected to control the algal community by causing changes in the density and traits of the limpet. In the bottom photograph, *Lithoderma* is seen as a black area around the limpets, and *Ulva* grows in the light-colored areas around *Lithoderma*.

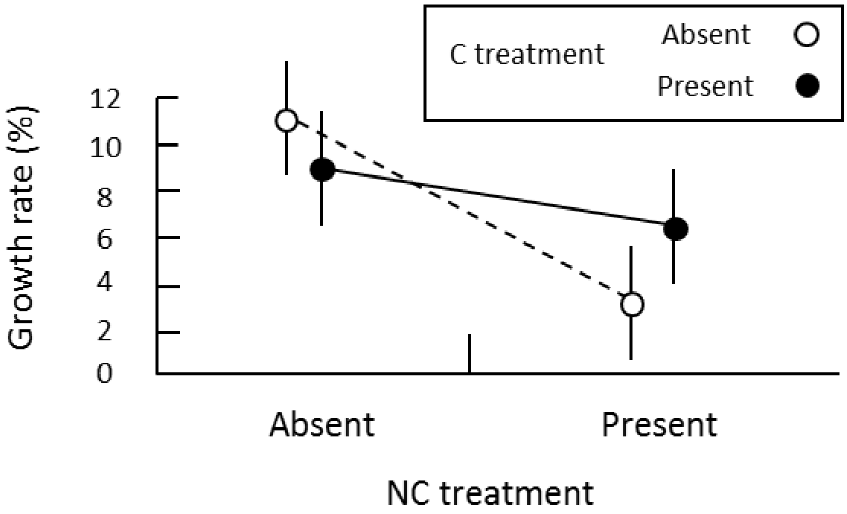

Fig. 2. Growth rate of limpets (*Siphonaria sirius*) in the presence/absence of the consumptive treatment and non-consumptive treatment. Responses are shown as mean ± SE of four replicates within each treatment. C and NC represent the consumptive and non-consumptive treatments, respectively.

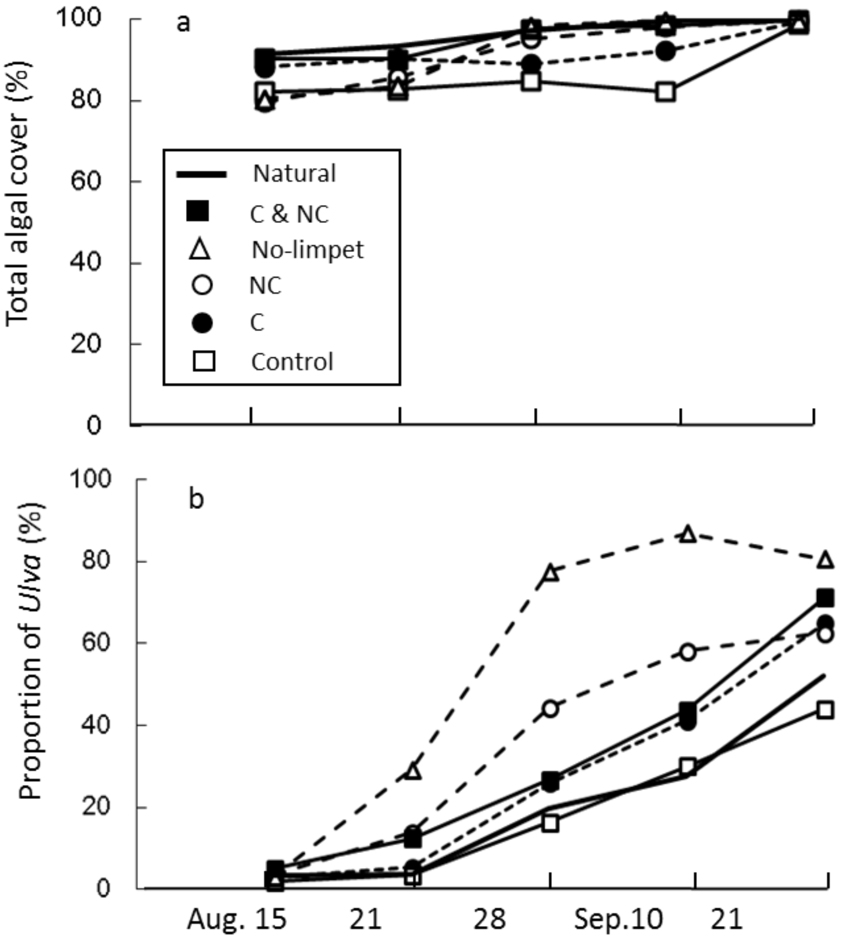

Fig. 3. (a) Temporal changes in the total percent cover of algae (*Ulva*, *Lithoderma*, and other minor algae) in each treatment plot. (b) Temporal changes in the percentage of *Ulva* in the total algal coverage. C and NC represent the consumptive and non-consumptive treatments, respectively.

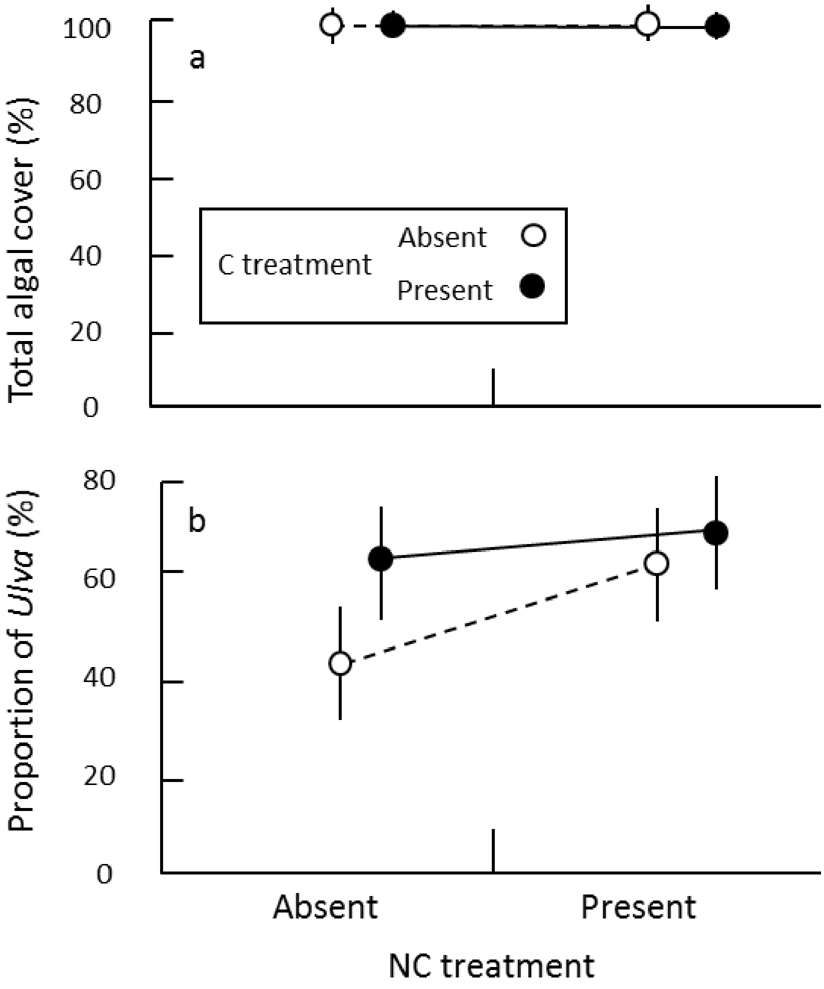

Fig. 4. (a) Final total percent cover of algae in the presence/absence of the consumptive treatment (i.e., density-mediated indirect interactions, DMIIs) and non-consumptive treatment (trait-mediated indirect interactions, TMIIs). Responses are shown as mean ± SE of four replicates within each treatment. (b) Percentage of *Ulva* in the total algal coverage. C and NC represent the consumptive and non-consumptive treatments, respectively.

TABLES

Table 1. Results of general linear model on the effects of consumptive (C) and non-consumptive (NC) treatments, the mean tidal level, and their interaction terms on the limpet's (*Siphonaria sirius*) traits. *P*-values below 0.05 are underlined.

	Growth		Disappeared				Feeding rate			
	Entire period		Initial 1 week		Entire period		Initial 1 week		Entire period	
Source	$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P
CE	0.05	0.83	1.50	0.26	1.95	0.20	0.33	0.58	1.56	0.25
NCE	9.07	0.02	6.89	0.03	11.48	0.01	10.46	0.01	1.31	0.29
Tidal level	5.09	0.054	1.19	0.31	8.47	0.02	3.17	0.11	0.14	0.72
CE×NCE	2.17	0.18	0.05	0.83	0.04	0.86	0.03	0.88	0.35	0.57
Tidal level×CE	0.31	0.59	0.35	0.57	9.70	0.01	3.09	0.12	0.19	0.67
Tidal level×NCE	0.64	0.45	0.34	0.58	0.05	0.83	1.86	0.21	0.98	0.35
Tidal level×CE×NCE	0.59	0.46	3.50	0.10	0.07	0.80	0.03	0.88	0.58	0.47

Table 2. Results of general linear model on the strengths of density-mediated indirect interactions (DMIIs), trait-mediated indirect interactions (TMIIs), the effects of mean tidal level, and their interaction terms towards the total percent cover of algae and percentage of *Ulva* in the total algal coverage. *P*-values below 0.05 are underlined.

	Total cover				Proportion of *Ulva* sp.			
	Initial 1 week		Entire period		Initial 1 week		Entire period	
Source	$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P
DMII	0.79	0.40	0.37	0.56	0.01	0.93	6.76	0.03
TMII	0.002	0.10	0.06	0.81	10.57	0.01	6.29	0.04
Tidal level	1.50	0.26	1.23	0.30	1.14	0.32	1.11	0.32
DMII×TMII	0.07	0.80	0.02	0.90	0.37	0.56	4.10	0.08
Tidal level×DMII	0.20	0.67	0.77	0.41	0.69	0.43	0.01	0.92
Tidal level×TMII	1.23	0.30	0.04	0.84	0.82	0.39	0.74	0.41
Tidal level×DMII×TMII	0.002	0.96	<0.001	0.998	0.48	0.51	0.34	0.58

Table 3. The principal results of the present study.

	Consumptive / density-mediated		Non-consumptive / trait-mediated	
	1 week	Final	1 week	Final
a) Direct effects				
Growth		0		−
Disappeared	0	0	+	+
Feeding rate	0	0	−	0
b) Indirect interactions				
Total cover	0	0	0	0
Proportion of *Ulva* sp.	0	+	+	+

Notes: Effects are consumptive or non-consumptive for direct effects, density-mediated, or trait-mediated for indirect interactions. Plus signs (+) indicate positive effects, minus signs (-) indicate negative effects, and zero (0) signs indicate the lack of significant effects. Blank cells indicate that no data were available.

APPENDIX DATA

Appendix figures

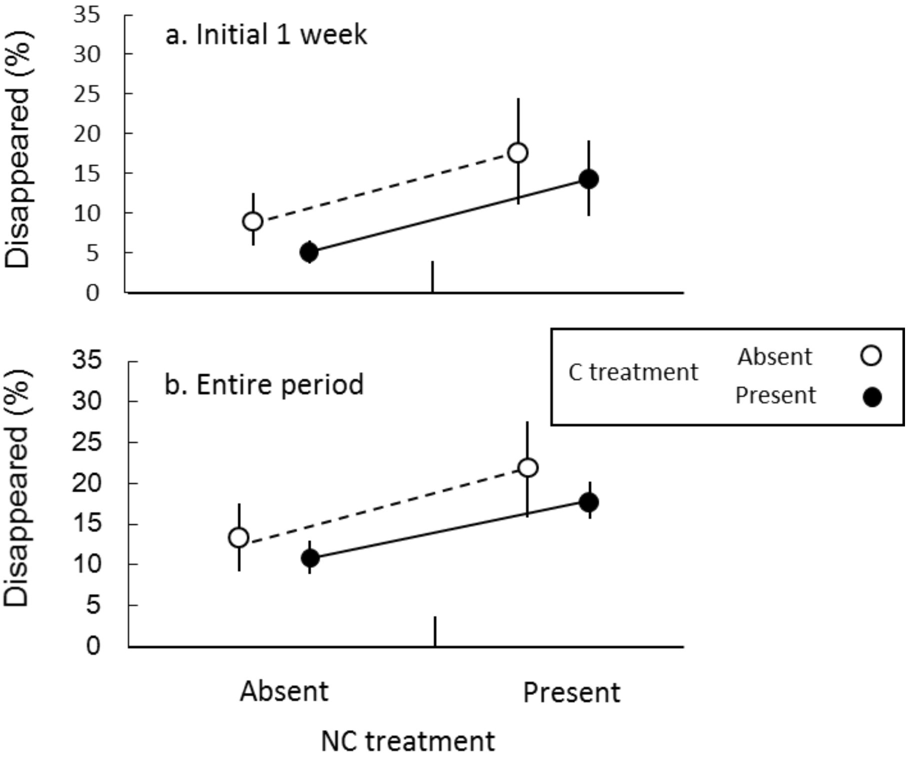

Fig. A1. Proportion of limpet individuals that disappeared in the presence/absence of the consumptive treatment and non-consumptive treatment, in the initial 1 week (a), and the average over the entire period (b). Responses are shown as mean ± SE of four replicates within each treatment. C and NC represent the consumptive and non-consumptive treatments, respectively.

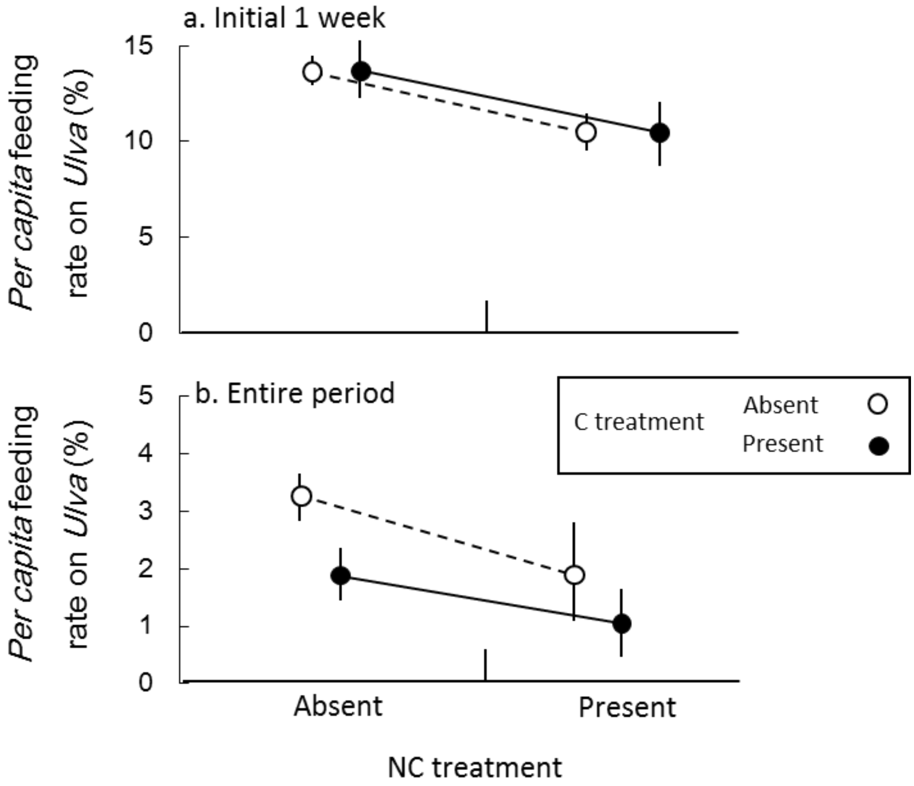

Fig. A2. *Per capita* feeding rate of the limpet on the alga *Ulva* in the presence/absence of the consumptive treatment and non-consumptive treatment, in the initial 1 week (a), and the average over the entire period (b). Responses are shown as mean ± SE of four replicates within each treatment. C and NC represent the consumptive and non-consumptive treatments, respectively.

Appendix tables

Table A1. Effects of copper paint under consumptive or non-consumptive treatment, and effects of setting cages with epoxy putty on the various characteristics of limpets.

	Consumptive treatment		Non-consumptive treatment		Cage	
	With copper (A)	Without copper (B)	With copper (C)	Without copper (D)	Present (E)	Absent (F)
Growth rate (%)	4.31	3.00	1.65	1.42	4.06	4.88
	t = 1.40, *P* = 0.29		*t* = 0.16, *P* = 0.88		*t* = -0.23, *P* = 0.83	
Disappeared (%)	2.56	10.26	17.78	23.24	18.41	19.28
	t = -0.97, *P* = 0.40		*t* = -0.56, *P* = 0.61		*t* = -0.20, *P* = 0.85	
Activity (%)	10.22	11.21	26.64	25.45	9.2	8.66
	t = -0.06, *P* = 0.96		*t* =0.05, *P* = 0.96		*t* = -0.003, *P* = 0.998	
Immigration rate (%)	0.00	0.00	0.00	0.00	–	–
Emigration rate (%)	0.00	1.11	0.00	1.11	0.00	0.00
	t = -1.00, *P* = 0.42		*t* = -1.00, *P* = 0.42			

Methods: I conducted experiments from 19 August through 2 September 2012 (14 days). I selected six rocks in each of three sites where I conducted the main experiment (A – F; 18 in all). The edges of plots A, C, E and F were lined with copper paint. I examined the effects of copper paint on limpets under consumptive treatment using plots A and B, and those under non-consumptive treatment using plots C and D, and effects of setting cages with epoxy putty on limpets outside the cages using plots E and F ($n = 3$ replicates). The consumptive and non-consumptive effects were conducted using the same methods as in the main experiment, based on the predation rates estimated in 2010. Fifteen randomly selected limpets were individually marked both inside and outside of each of plots A – D, and only limpets inside were marked for plots E and F, as setting cages is unlikely to affect immigration. The growth rate, proportion of marked limpets that disappeared, etc. were checked using the same methods as in the main experiment. I analyzed the data with paired *t*-test after arcsine transformation, except for growth rate (which was untransformed).

Table A2. Results of ANOVA on the strengths of DMIIs, TMIIs and their interaction term towards the amounts of chlorophyll *a*, chlorophyll *b* and chlorophyll *a* + *b* per 3 cm^2.

	Chlorophyll *a*				Chlorophyll *b*				Chlorophyll *a* + *b*			
	Initial 2 weeks		Entire period		Initial 2 weeks		Entire period		Initial 2 weeks		Entire period	
Source	$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P
DMII	1.87	0.21	4.33	0.07	1.17	0.31	0.97	0.35	2.04	0.19	4.02	0.08
TMII	0.002	0.96	2.46	0.16	0.01	0.77	0.09	0.77	0.01	0.91	1.78	0.22
Tidal level	0.61	0.46	1.08	0.33	1.05	0.34	0.005	0.94	1.04	0.34	0.61	0.5
DMII × TMII	0.10	0.76	4.44	0.07	0.17	0.70	1.95	0.20	0.17	0.69	4.78	0.06
Tidal level × DMII	1.81	0.22	2.06	0.19	0.001	0.98	1.33	0.28	0.74	0.41	2.47	0.15
Tidal level × TMII	0.90	0.37	0.20	0.66	0.60	0.46	0.25	0.63	1.01	0.34	0.30	0.60
Tidal level × DMII × TM	1.09	0.33	0.03	0.87	0.004	0.95	0.30	0.60	0.51	0.50	0.12	0.74

CHAPTER 3

Prey Density Affects Strengths of Density- and Trait-mediated Indirect Interactions of Predators on an Algal Community

Abstract. In trophic cascades, predators can indirectly affect resources by reducing prey density (density-mediated indirect interactions; DMIIs) or by changing prey traits, such as their behavior, morphology, physiology, or life history (trait-mediated indirect interactions; TMIIs). Although the importance of predator (e.g., foraging strategy) and resource characteristics (e.g., quantity) in these indirect interactions is well recognized, little attention has been paid to prey characteristics (e.g., density), especially in field studies. I focused on a marine trophic cascade involving the carnivorous snail *Thais clavigera*, its limpet prey *Siphonaria sirius*, and the algae *Lithoderma* sp. and *Ulva* sp. Using intertidal rocks as natural replicates, I experimentally evaluated the *in situ* effects of the DMIIs and TMIIs on the algal community under two density ranges of prey. The strengths of consumptive effects (CEs) and non-consumptive effects (NCEs) of the predator on the prey limpet were also monitored to elucidate the mechanisms of the indirect interactions. At high densities, CEs decreased the percentage of individual limpets that disappeared (that likely died); however, neither DMIIs nor TMIIs were detected. At low densities, both CEs and NCEs reduced the per capita feeding rate and CEs reduced the growth rate of limpets. Moreover, *Lithoderma* sp. was replaced by *Ulva* sp. through both DMIIs and TMIIs. These results suggest that prey density is a key determinant of the strengths of CEs and NCEs, and notably, of DMIIs and TMIIs.

INTRODUCTION

Ecosystems are among the most complicated systems in nature (Schmitz 2010). For instance, in many terrestrial and aquatic communities, predators can influence the abundance, distribution, and interactions of various organisms through top-down control (Schmitz 2010). Such trophic cascades initiate when predators reduce the density of prey (consumptive effects or CEs) or alter prey behavior, morphology, physiology, or life history (non-consumptive effects or NCEs) (Lima 1998). In turn, CEs and NCEs on prey influence basal resource via density- (DMIIs) and trait-mediated indirect interactions (TMIIs), respectively (Trussell et al. 2006, Schmitz 2010, Ohgushi et al. 2012). To unveil the mechanisms that maintain the complicated ecosystems, evaluating the strengths of such indirect interactions and identifying the factors that affect them are key issues (Ohgushi et al. 2012).

Several potential factors affecting the strengths of DMIIs and TMIIs have been identified, including the foraging mode of predators (Henry et al. 2010, Schmitz 2010) and the quality and quantity of resources (Luttbeg et al. 2003, Wojdak and Luttbeg 2005, Trussell et al. 2008). The habitat type of prey (risky vs. safe, or simple vs. complex) is also known to affect the strength of TMIIs relative to DMIIs (Trussell et al. 2006, Schmitz 2010, Alexander et al. 2013). However, little attention has been given to the role of the prey's own characteristics such as density, size, or experience (Matassa and Trussell 2014). This is surprising given the fact that prey mediate the top-down indirect interactions of predators on resource quality, quantity, and even community structure (Schmitz 2010, Ohgushi et al. 2012, Wada et al. 2013).

Prey density may be an important characteristic in determining the strengths of indirect interactions (Holt and Barfield 2012) because it can influence the strength of CEs through the number of predator－prey encounters (Turesson and Brönmark 2007) or the density-dependent life history traits of the prey such as survival, growth, or reproduction (e.g., Yoshida et al. 2013 in gastropods). The extent of NCEs may also depend on the prey's density (Peacor and Werner 2000, Relyea 2004, Turner 2004, Belovsky et al. 2011, Yoshie and Yusa 2011) as a result of changes in the optimal trade-off between feeding and avoiding predation. In fact, Hughes et al. (2012) showed that the density of prey mud crabs *Panopeus* spp. affected the strengths of the indirect interactions between the predator hardhead catfish *Ariopsis felis* and the oyster *Crassostrea virginica* in short-term (4 days) mesocosm experiments.

Furthermore, prey density often varies in an unpredictable way. This is especially true in marine ecosystems, where variation in larval recruitment or post-settlement mortality is a determinant of the adult population density (Underwood and

Fairweather 1989, Caley et al. 1996). Therefore, it is reasonable to treat prey density as an independent factor that can affect the strengths of indirect interactions, and hence the community structure (Wada et al. 2013), rather than a factor merely affected by the predation or resource availability.

Largely due to the difficulty of maintenance of experiments, TMIIs and the factor that influences them have been typically studied in closed laboratory experiments or mesocosms, where odors from feeding predators tend to be retained in the study system for a long duration. However, in nature, especially in marine environments, odor from a predator may be diluted or washed away sooner. Moreover, the strengths of indirect interactions vary with the duration of observation (Luttbeg et al. 2003, Abrams 2008, Hoverman and Relyea 2012). Therefore, evaluating the role of prey density in DMII and TMII strengths requires a long-term field experiment. However, no such studies seem to exist in marine ecosystems.

Here, I investigated *in situ* how CEs and NCEs and the subsequent DMIIs and TMIIs operate at two different density ranges of the prey. The study system consisted of the carnivorous muricid snail *Thais clavigera*, its prey the pulmonate limpet *Siphonaria sirius*, and the prey resources the green alga *Ulva* sp. and the cyanobacterium (blue-green “alga”) *Lithoderma* sp. (Wada et al. 2013). The limpet has a home range in which it preferentially forages competitively dominant *Ulva* over subordinate *Lithoderma* and shows an escape behavior when attacked by the predatory snail (Iwasaki 1993a). I hypothesized that the strengths of DMIIs and TMIIs, as well as strengths of CEs and NCEs, differ between the two density ranges of the limpet. When the density is high, I would expect that both the CEs and DMIIs are weak because competition between individuals is intense and the direct consumption by the predator will result in the relaxation of the competition. Likewise, at high limpet density the NCEs and TMIIs are also expected to be weak if the limpets exhibit less intense anti-predator responses under low food availability per individual, as known in many gastropods ("hunger overcomes fear"; Morton and Chan 1999, Matassa and Trussell 2014). In contrast, at low limpet densities, I would expect that both consumptive and non-consumptive predator effects will result in the changes in algal community structure via DMIIs and TMIIs, respectively, as I reported previously (Wada et al. 2013).

MATERIALS AND METHODS

Experimental plots

I conducted the field experiment, modified from Wada et al. (2013), near Seto Marine Biological Laboratory, Wakayama, Japan (33.75° N, 135.28° E). The experiment spanned 29 days, from 15 August to 13 September 2011. Censuses were made four times at intervals of 7 – 13 days. During the season including this period of the experiment, *S. sirius* actively forages and reproduces, but larval recruitment does not occur (Iwasaki 1993b, Iwasaki 1995a, 1995b). Moreover, the green alga *Ulva* sp. increases in cover from late summer to winter (Iwasaki 1993b).

I selected 36 sandstone rocks located at least 0.8 m apart in the lower intertidal area (at depths of －51 to －30 cm relative to the mean tidal level) where *S. sirius* was the dominant herbivore and *T. clavigera* was the major predator on *S. sirius*. The uppermost side of each rock was used as the experimental plot. The edge of each plot was lined (~ 5 cm width) with paint containing copper powder (Denka, Tokyo, Japan) to prevent most benthic animals from moving into or out of the plots (Cubit 1984). There are no effects of the paint on limpet survival, growth, or activity, and the limpets do not migrate between the plots even without the copper paint (Wada et al. 2013). I removed all the herbivores (except for *S.sirius*) and carnivores from the experimental plots at the beginning of and during the experiment whenever found. The sizes of experimental plots did not differ significantly among the four treatment groups (see "*Experimental treatments*" below) (two-way ANOVA; $F_{3, 27} = 1.74$, $P = 0.18$) or between two density ranges ($F_{1, 27} = 1.39$, $P = 0.25$).

Density ranges

In natural density ranges of the limpets, I selected 16 high- and 16 low-density plots from the 36 plots. In addition, I established four “no-limpet” plots, where all *S. sirius* were removed throughout the experiment, to observe changes in the algal community in the absence of limpets. To prepare the low-density plots, I removed 30.8 ± 16.3% (mean ± SD) of the limpets from nine plots with moderate limpet densities on 4 July 2011, more than 40 days before the experiment started to minimize any effects of the removal. The remaining seven low-density plots and all 16 high-density plots received no density manipulations beforehand. I kept the initial density manipulation at a minimum because i) I was interested in the role of prey density under natural conditions and ii) the limpets use the home scars that perfectly fit their shell shapes and hence transplanting was not feasible. Consequently, the limpet densities at the start of the experiment were 302.4 ± 100.2 individuals m^{-2} (mean ± SD, n = 16) in the high-density plots and 114.0 ± 51.8 individuals m^{-2} (n = 16) in the low-density plots. As expected, the difference in the density was significant between two density ranges (two-way ANOVA; $F_{1, 27} = 43.20$, $P < 0.001$), although it was not significant among the four treatment groups (see “*Experimental treatments*” below) ($F_{3, 27} = 0.68$, $P = 0.57$). The population size of *S. sirius* (i.e., total number of limpets in one plot) also varied among the experimental plots (12 – 259 individuals), and it differed between two density ranges (two-way ANOVA; $F_{1, 27} = 5.48$, $P = 0.03$). However, it did not differ significantly among the four treatment groups ($F_{3, 27} = 1.46$, $P = 0.25$).

A maximum of 30 randomly selected individuals of *S. sirius* (with shell lengths ranging from 8.3 – 28.2 mm and 9.3 – 24.8 mm in the high- and low-density plots, respectively) were individually marked with paint in each plot. The paint had no apparent effects on the behavior and survival of the limpets (Wada et al. 2013). Although there was a negative correlation between density and the average shell length of the limpets at the start of the experiment ($r = -0.44$, $P = 0.01$, $N = 32$ plots), the shell lengths did not differ significantly between the two density ranges (two-way ANOVA; $F_{1, 27} = 3.39$, $P = 0.08$) or among the four treatment groups ($F_{3, 27} = 0.05$, $P = 0.98$).

Experimental treatments

Following the standard experimental design (e.g., Peacor and Werner 2001, Griffin and Thaler 2006, Trussell et al. 2006, Wada et al. 2013), I applied two treatments to the limpets. First, a “consumptive treatment” was applied to estimate the strengths of the CEs of the snail predators on the prey limpets without predator odors and resulting DMIIs. I removed a fixed proportion of both marked and unmarked limpets with a scraper at each census (Wada et al. 2013); there was no apparent damage to the algae by this procedure. This proportion (4 – 12%; average 8.0% per census) was chosen based on the natural predation rates of limpets observed over the same season in the previous year (Wada et al. 2013), because the annual variation of the limpet mortality is small around the study area (Iwasaki 1993b). I applied the same natural predation rates to both density ranges because there was no evidence of density-dependent predation; for instance, no significant correlation was found between the limpet density and the predation rate, both of which were measured in nine unmanipulated plots and averaged over four censuses in August – September 2013 ($r = 0.31$, $P = 0.41$, $N = 9$).

Second, a “non-consumptive treatment”, in which odors from caged feeding predators were released, was used to quantify the strengths of the NCEs and TMIIs. For the treatment, I attached a small mesh cage (25 mm tall, 77 mm in diameter) using water-resistant epoxy glue (Konishi Corporation, Osaka, Japan) to the middle of the upper edge of each plot. I placed the predator *T. clavigera* (“C type” sensu [Abe, 1985]; shell height 19.1 – 26.3 mm) and the prey *S. sirius* (individuals previously removed from the consumptive plots) into each cage within all plots receiving the non-consumptive treatment to permit the release of odors of *T. clavigera* feeding on *S. sirius*. I did not distinguish the effects of odors released by the predators and by injured prey. The number of predator individuals (8 – 11 dependent on time) was based on the predator numbers observed in the same season in 2010 (Wada et al. 2013). The predator number was the same in all the plots with non-consumptive treatment because there was no correlation between the number of predators and plot size at the beginning of the experiment (r = 0.24, P = 0.16, N = 36). The proportion of limpet individuals placed in each odor cage to the total number of limpets in each plot was the same among the plots and was based on the predation rate in 2010 (i.e., the same proportion as that removed in the consumptive treatment). Therefore, the number of limpets given to the caged predators was proportional to the limpet population size in the plot, which will underestimate the effect of limpet population size (and hence density) on NCEs and TMIIs if the number of limpets actually eaten does not increase linearly with population size. The prey individuals were introduced in the odor cage after each census, and the survival of the predators in the cages was checked occasionally and dead individuals were replaced with new ones.

These treatments were also applied in combination, resulting in four treatment groups: consumptive only, non-consumptive only, both treatments, and neither treatment (control). The four treatment groups were randomly allocated within both 16 high- and 16 low-density plots (each with four replicates). I also attached the cages to the plots with control or consumptive-only treatments but no predators or limpets were placed in the cages.

Estimation of direct effects and indirect interactions

To estimate the CEs and NCEs on *S. sirius*, I calculated the *S. sirius* growth rate, the percentage of individuals that disappeared, and the per capita feeding rate on *Ulva* sp. for each plot. The growth rate was expressed as the final shell length of each marked limpet relative to the initial shell length. Shell lengths were measured on 15 and 28 August and 25 September. I was unable to measure the shell lengths of all marked limpets on the final day of the experiment (13 September) because of high tides caused by a typhoon. Therefore, I continued the experimental treatments up to 25 September and estimated the shell length on the final day (13 September) from the measurements on 28 August and 25 September under the assumption of linear growth. The estimated values were highly correlated with the actual values for the individuals that could be measured on 13 September ($r = 0.99$, $P < 0.0001$, $N = 114$).

To calculate the percentage of individuals that disappeared (and were likely dead because the limpets seldom left their home ranges; Wada et al. 2013), the number of individuals missing at each census was divided by the number of survivors at the previous census. The number of the limpets removed in consumptive treatment was not included in the calculation. This proportion was measured twice, on 29 August and 13 September, and averaged.

The per capita feeding rate of limpets on *Ulva* sp. (i.e., the average quantity of *Ulva* eaten per *S. sirius* individual per day) was calculated as the mean percent cover of *Ulva* sp. (see the following paragraph) in the no-limpet plots minus the percent cover of *Ulva* sp. in each treatment plot, divided by the cumulative number of limpets in the plot over the course of the experiment. The cumulative number of limpets was calculated as the number of individuals that survived to each census date × the number of days from the start of the experiment to the census (Wada et al. 2013). The per capita feeding rate on *Lithoderma* was not calculated because the amount of cover decreased in the absence of limpets.

To estimate the strengths of DMIIs and TMIIs on algae, I measured the percent cover of each algal species. The percent cover is likely to reflect the actual algal quantity because chlorophyll content (chlorophyll *a*, *b*, or *a* + *b*) per unit area was not different among treatments on this time scale (Wada et al. 2013). To calculate the percent cover, I placed two quadrats (18.5×18.5 cm) just below and to the right of the cage and photographed them. I plotted 169 equidistant points within each quadrat on a PC and counted the number of points that were superimposed on each algal species. This procedure was repeated four times during the observation period (on 16, 22, and 28 August and 12 September), and all the values (two quadrates over four times) were averaged.

Statistical analyses

Because I was interested in the strengths of direct effects (CEs and NCEs) and indirect interactions (DMIIs and TMIIs) at each density range, the data from the high- and low-density experiments were analyzed separately. Then, to statistically compare the effect sizes (see below) of direct and indirect interactions between the two density ranges, I used a two-sample *t*-test. As compared with the statistical model incorporating the limpet density as an explanatory variable, the present model has merits that i) the effects of consumptive and non-consumptive treatments can be detected as main effects rather than the interaction terms between density and the treatments, and ii) their strengths can be directly compared between the high- and low-density ranges.

To test for the effects of treatments on the each characteristic of limpets and algae, I included the presence/absence of the consumptive and non-consumptive treatments and their interaction term. Moreover, the mean tidal level of each experimental plot, as well as its interaction terms with consumptive and non-consumptive treatments, was also included in the model to statistically control the effects of tidal level (Wada et al. 2013). Furthermore, considering the great variation, I included the population size of the limpet as a covariate. All explanatory variables were treated as fixed factors, and proportion data were logit-transformed after adding 0.005 to meet the assumptions of the statistical model (Warton and Hui 2011). Analyses were conducted using JMP version 11 (SAS Institute, Cary, North Carolina, USA).

Effect size

I compared the CEs or NCEs on growth rate of limpets between the two density ranges, using a ratio-based approach (Trussell et al. 2006) as:

$\mathrm{CE} = 1 - G_{\text{consumptive only}} / G_{\text{control}}$

$\mathrm{NCE} = 1 - G_{\text{non-consumptive only}} / G_{\text{control}}$.

For these proportions, the numerator was the growth rate (G) in each replicate for consumptive only or non-consumptive only treatment of either high- or low-density range, whereas the denominator was the average growth rate of four control plots of the same density range (see Trussell et al. 2006 for a similar approach). Similarly, magnitudes of CEs and NCEs on the percentage of disappeared individuals and on the feeding rate of limpets were calculated and compared between density ranges.

Similarly, I calculated effect sizes for the DMIIs or TMIIs of predators on percent cover of *Ulva* sp. (C) following Trussell et al. (2006) as:

$\mathrm{DMII} = C_{\text{consumptive only}} / C_{\text{control}} - 1$

$\mathrm{TMII} = C_{\text{non-consumptive only}} / C_{\text{control}} - 1$.

The numerator was provided by each replicate, whereas the denominator was the average of the four control plots.

RESULTS

The tidal level, including its interaction terms with the treatment, and the population size of limpets did not significantly affect the limpets' (i.e., direct effects) or algal traits (indirect interactions) (Table 1). Therefore, in the following I focus on the effects of consumptive and non-consumptive treatments on these traits.

Direct effects on limpets

At high densities of limpets, no effects of the consumptive treatment, non-consumptive treatment, or their interaction were detected on the growth rate of limpets over the 29-day experiment (Table 1a, Appendix: Fig. A1a). However, the consumptive treatment reduced the growth rate at low densities of limpets (Appendix: Fig. A1b), although neither the non-consumptive treatment nor the interaction term affected the growth rate (Table 1a).

The percentage of *S. sirius* individuals that disappeared decreased under the consumptive treatment but not under the non-consumptive treatment at high densities (Fig. 1a, Table 1a). In particular, the average percentage of disappearance was 15.2%

whereas it was 7.7% in plots with the consumptive treatment (i.e., a 7.5% increase). In contrast, at low densities, no treatment or interaction effects were detected on the percentage which disappeared (Fig. 1b, Table 1a).

At high densities, there were no treatment or interaction effects on the per capita feeding rate of *S. sirius* on the green alga *Ulva* sp. (Fig. 2a, Table 1a). However, at low densities, both the consumptive and the non-consumptive treatments reduced the per capita feeding rate (Fig. 2b, Table 1a). The average feeding rate of the control plots was 7.3% at high densities and 14.3% at low densities.

Indirect interactions with algae

The two species of algae covered most rock surfaces (ca. 90%) in the plots throughout the experimental period at both densities (Appendix: Fig. A2). Moreover, the percent cover of *Ulva* sp. increased over time in all treatment groups at both densities (Appendix: Fig. A3). In contrast, the coverage of *Lithoderma* sp. decreased over time. Overall, the changes at low densities were more pronounced than those at high densities.

The consumptive treatment, non-consumptive treatment, or their interaction had no effects on the total algal cover at either high or low densities (Table 1b; Appendix: Fig. A4). Moreover, these treatments did not affect the percent cover of *Ulva* at high densities (Fig. 3a, Table 1b). In contrast, both DMIIs and TMIIs increased the percent cover of *Ulva* at low densities (Fig. 3b, Table 1b).

Effect size

For direct interactions, the CEs on the growth rate were stronger at low densities than at high densities (Table 2). Similarly, the NCEs on the per capita feeding rate were stronger at low than high densities. No other differences were detected for other direct effects.

For indirect interactions, the effect sizes of DMIIs and TMIIs on the total algal cover did not differ between density ranges (Table 2). However, both DMIIs and TMIIs on the percent cover of *Ulva* were stronger at low densities than at high densities.

DISCUSSION

The strengths of the DMIIs and TMIIs differed between two natural density ranges of the limpet *S. sirius* (Table 3), supporting my hypothesis that prey density is a key factor transmitting top-down indirect interactions. In particular, both the DMIIs and TMIIs affected the community structure of the algae in terms of the percent cover of *Ulva* sp. at low limpet densities, whereas neither effect was detected at high densities. To my knowledge, this is the first long-term field study showing the role of prey density in transmitting top-down indirect interactions in a marine ecosystem.

Direct effects

To understand the mechanisms of indirect interactions, the nature of direct effects should be considered. The consumptive treatment reduced the percentage of individuals that disappeared at high densities and not low densities. Because individual *S. sirius* maintain home ranges in which they forage (Ohgushi et al. 1953) and emigration from the plots was negligible in the study area (Wada et al. 2013), this result suggests that limpets experienced lower mortality under the consumptive treatment at high than low densities. This may be due to reduced intraspecific competition by simulated predation. In fact, many predators reduce competition among prey individuals in marine ecosystems (e.g. Trussell et al. 2006).

At low densities, the limpets exposed to odors of predatory *T. clavigera* reduced their feeding rate, as reported previously (Wada et al. 2013). In contrast, at high densities, there was no effect of the non-consumptive treatment on the feeding rate. Food is more limited per individual limpet when density is high (Fig. 2), and therefore limpets might have maintained their feeding activity even in the presence of foraging predator odors, as known in other gastropods (“hunger overcomes fear”; Morton and Chan 1999, Turner 2004, Matassa and Trussell 2014).

The consumptive treatment also reduced per capita feeding rate and growth rate of the limpets at low densities and not at high densities. This result is rather unexpected because the consumptive treatment reduced the limpet density, and thereby could have improved the food availability and growth rate. However, at low densities, *Ulva* sp. increased greatly by the consumptive treatment during the experiment (Appendix: Fig. A3b). It is possible that overgrown algal thalli became foliose and might have impeded the limpets' efficient grazing (Jenkins et al. 1999).

Density affects parameters of prey population such as mortality and growth (Seitz et al. 2001, Turner 2004, Yoshie and Yusa 2011). Moreover, the effects of anti-predator behavior (i.e., non-consumptive effect) on survival and reproduction of prey vary with prey density (Belovsky et al. 2011). In this study, I showed that the strengths of both consumptive and non-consumptive predator effects on the traits of prey depend on prey density. Because CEs and NCEs drive various indirect interactions, prey density is crucial for understanding the mechanisms of ecosystem organization.

Indirect interactions

Both DMIIs and TMIIs increased the percentage cover of *Ulva* sp. and decreased that of *Lithoderma* sp. at low limpet densities, whereas neither did at high densities (Table 3). As a result, there was a significant difference in effect sizes of

DMIIs and TMIIs between two density ranges. The TMIIs affected algal community structure only at low densities because the non-consumptive effects on the per capita feeding rate were present only when prey density was low. Similarly, detection of DMIIs only at low limpet densities is consistent with the reduced feeding rate by the consumptive treatment. Moreover, at low densities, the consumptive treatment had no significant effect on the rate of the limpet disappearance, whose value under the consumptive treatment (8.1 %) was similar to that in the control plots (10.9 %; Fig. 1b); thus, the consumptive treatment (i.e., scheduled removal) readily resulted in the density reduction, and hence, the change in algal community structure. In contrast, the scheduled removal (on average 8.0%) was compensated by increased ‘survival’ (a 7.5% increase) of the remaining limpets at high densities (Fig. 1a).

In this study, limpet density was negatively correlated with body size. Body size is an important factor affecting the strengths of feeding rates or anti-predator responses in gastropods (Ichinose 2002, Yoshida et al. 2013). However, body size is not a main factor affecting the strengths of direct effects and indirect interactions in this study because it did not differ significantly between high- and low-density plots. In addition, the anti-predator behavior of limpets attacked by *T. clavigera* is similar regardless of their sizes (Iwasaki 1993b).

Prey have an important role as an intermediate player in trophic chains, and they must balance the trade-off between foraging and predation risk. Therefore, the nature and degree of trophic control of ecosystems may be affected by way of how prey species balances the trade-off (Schmitz 2010). The present results suggest that prey density influences the strengths of DMIIs and TMIIs in modifying algal community structure via CEs and NCEs. Because densities of marine animals vary greatly both temporally and spatially, being affected by many factors such as climate or recruitment (Underwood and Fairweather 1989), it is probable that the strengths of indirect interactions vary accordingly. Therefore, in evaluating DMIIs and TMIIs, the importance of prey characteristics including density requires further exploration (Hughes et al. 2012, Holt and Barfield 2012), together with the characteristics of predators and resources.

LITERATURE CITED

Abe, N. 1985. Two forms of *Thais clavigera* (Küster, 1858). Venus (the Japanese journal of malacology) 44:15-26.

Abrams, P. A. 2008. Measuring the impact of dynamic anti-predator traits on predator-prey -resource interactions. Ecology 89:1640-1649.

Alexander, M. E., J. T. A. Dick, and N. E. O'Connor. 2013. Trait-mediated indirect interactions in a marine intertidal system as quantified by functional responses. Oikos 122:1521-1531.

Belovsky, G. E., A. N. Laws, and J. B. Slade. 2011. Prey change behaviour with predation threat, but demographic effects vary with prey density: experiments with grasshoppers and birds. Ecology Letters 14:335-340.

Caley, M. J., M. H. Carr, M. A. Hixon, T. P. Hughes, G. P. Jones, and B. A. Menge. 1996. Recruitment and the local dynamics of open marine populations. Annual Review of Ecology and Systematics 27:477–500.

Cubit, J. D. 1984. Herbivory and the seasonal abundance of algae on high intertidal rocky shore. Ecology 65:1904-1917.

Griffin, C. A. M., and J. S. Thaler. 2006. Insect predators affect plant resistance via density- and trait indirect interactions. Ecology Letters 9:338-346.

Henry, L. M., J. A. Bannerman, D. R. Gillespie, and B. D. Roitberg. 2010. Predator identity and the nature and strength of food web interactions. Journal of Animal Ecology 79:1164-1171.

Holt, R. D., and M. Barfield, 2012. Trait-mediated effects, density dependence and dynamic stability of ecological systems. In: Ohgushi, T., Schmitz, O., and Holt, R.D.

(Eds), Trait-mediated indirect interactions. Cambridge University Press, Cambridge, pp. 89-106.

Hoverman, J. T., and R. A. Relyea, 2012. The long-term impacts of predators on prey: inducible defenses, population dynamics, and indirect effects. Oikos 121:1219-1230.

Hughes, A. R., K. Rooker, M. Murdock, and D. L. Kimbro. 2012. Predator cue and prey density interactively influence indirect effects on basal resources in intertidal oyster reefs. Plos One 7:e44839.

Ichinose, K. 2002. Influence of age and body size on alarm responses in a freshwater snail *Pomacea canaliculata*. Journal of Chemical Ecology 28:2017-2028.

Iwasaki, K. 1993a. Analyses of limpet defense and predator offense in the field. Marine Biology 116:277-289.

Iwasaki, K. 1993b. The role of individual variability in limpet resting site fidelity and competitive ability in the organization of a local rocky intertidal community. Physiology and Ecology Japan 30:31-70.

Iwasaki, K. 1995a. Foraging and spawning rhythms of the pulmonate limpet *Siphonaria sirius* (Pilsbry): switching of activity period by a diurnal forager. Journal of Molluscan Studies 61:275-288.

Iwasaki, K. 1995b. Dominance order and resting site fidelity in the intertidal pulmonate limpet *Siphonaria sirius* (Pilsbry). Ecological Research 10:105-115.

Jenkins, S. R., S. J. Hawkins, and T. A. Norton. 1999. Direct and indirect effects of a macroalgal canopy and limpet grazing in structuring a sheltered inter-tidal community. Marine Ecology Progress Series 188:81–92.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions: What are the ecological effects of anti-predator decision-making? BioScience 48:25-34.

Luttbeg, B., L. Rowe, and M. Mangel. 2003. Prey state and experimental design affect relative size of trait- and density-mediated indirect effects. Ecology 84:1140-1150.

Matassa, C. M., and G. C. Trussell. 2014. Prey state shapes the effects of temporal variation in predation risk. Proceedings of the Royal Society B 281:20141952

Morton, B., and K. Chan. 1999. Hunger rapidly overrides the risk of predation in the subtidal scavenger *Nassarius siquijorensis* (Gastropoda: Nassariidae): an energy budget and a comparison with the intertidal *Nassarius festivus* in Hong Kong. Journal of Experimental Marine Biology and Ecology 240:213-228.

Ohgushi, T., O. J. Schmitz, and R. D. Holt. 2012. Trait-mediated indirect interactions. Cambridge University Press, Cambridge.

Ohgushi, R., T. Miura, and M. Kotani. 1953. Some observations on the homing behaviors of two species of limpets. Venus (the Japanese journal of malacology)

17:219-223.

Peacor, S. D., and E. E. Werner. 2000. Predator effects on an assemblage of consumers through induced changes in consumer foraging behavior. Ecology 81:1998-2010.

Peacor, S. D., and E. E. Werner. 2001. The contribution of trait-mediated indirect effect to the net effects of a predator. Proceedings of the National Academy of Sciences USA 98:3904-3908.

Relyea, R. A. 2004. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. Ecology 85:172-179.

Schmitz, O. J. 2010. Resolving Ecosystem Complexity. Princeton University Press, Princeton.

Seitz, R. D., R. N. Lipcius, A. H. Hines, and D. B. Eggleston. 2001. Density-dependent predation, habitat variation, and the persistence of marine bivalve prey. Ecology 82:2435-2451.

Trussell, G. C., P. J. Ewanchuk, and C. M. Matassa. 2006. Habitat effects on the relative importance of trait- and density-mediated indirect interactions. Ecology Letters 9:1245-1252.

Trussell, G. C., P. J. Ewanchuk, and C. M. Matassa. 2008. Resource identity modifies the influence of predation risk on ecosystem function. Ecology 89:2798-2807.

Turesson, H., and C. Brönmark. 2007. Predator-prey encounter rates in freshwater piscivores: effects of prey density and water transparency. Oecologia 153:281-290.

Turner, A. M. 2004. Non-lethal effects of predators on prey growth rates depend on prey density and nutrient addition. Oikos 104:561-569.

Underwood, A. J., and P. G. Fairweather. 1989. Supply-side ecology and benthic marine assemblages. Trends in Ecology & Evolution 4:16-20.

Wada, Y., K. Iwasaki, and Y. Yusa. 2013. Changes in algal community structure via density- and trait-mediated indirect interactions in a marine ecosystem. Ecology 94:2567-2574.

Warton, D. I., and F. K. C. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3-10.

Wojdak, M. J., and B. Luttbeg. 2005. Relative strengths of trait-mediated and density-mediated indirect effects of a predator vary with resource levels in a freshwater food chain. Oikos 111:592-598.

Yoshie, H., and Y. Yusa. 2011. Indirect interactions in a rice ecosystem: density dependence and the interplay between consumptive and non-consumptive effects of predators. Freshwater Biology 56:302-310.

Yoshida, K., K. Hoshikawa, T. Wada, Y. and Yusa. 2013. Patterns of density dependence in growth, reproduction and survival in the invasive freshwater snail *Pomacea canaliculata* in Japanese rice fields. Freshwater Biology 58:2065-2073.

FIGURES

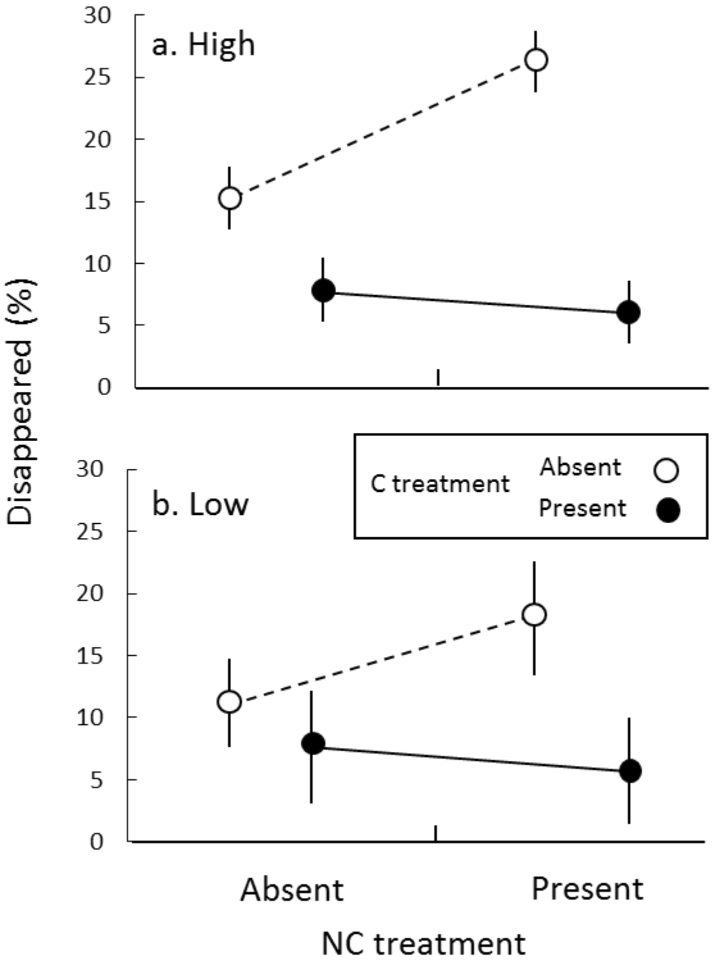

Fig. 1**.** Percentage of limpets, *Siphonaria sirius*, that disappeared in the presence/absence of the consumptive and non-consumptive treatments in (a) high-density and (b) low-density plots. Responses are shown as the mean ± SE of four replicates within each treatment. C and NC represent the consumptive and non-consumptive treatments, respectively.

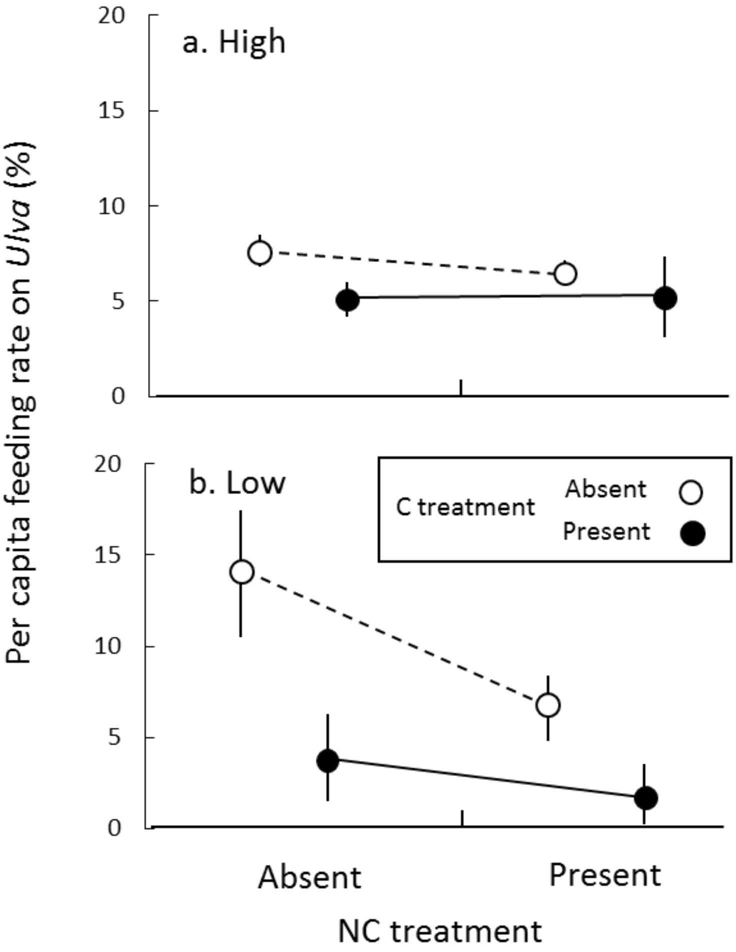

Fig. 2. Per capita feeding rate of limpets on the alga *Ulva* in the presence/absence of the consumptive and non-consumptive treatments in (a) high-density and (b) low-density plots. Responses are shown as the mean ± SE of four replicates within each treatment. C and NC represent the consumptive and non-consumptive treatments, respectively.

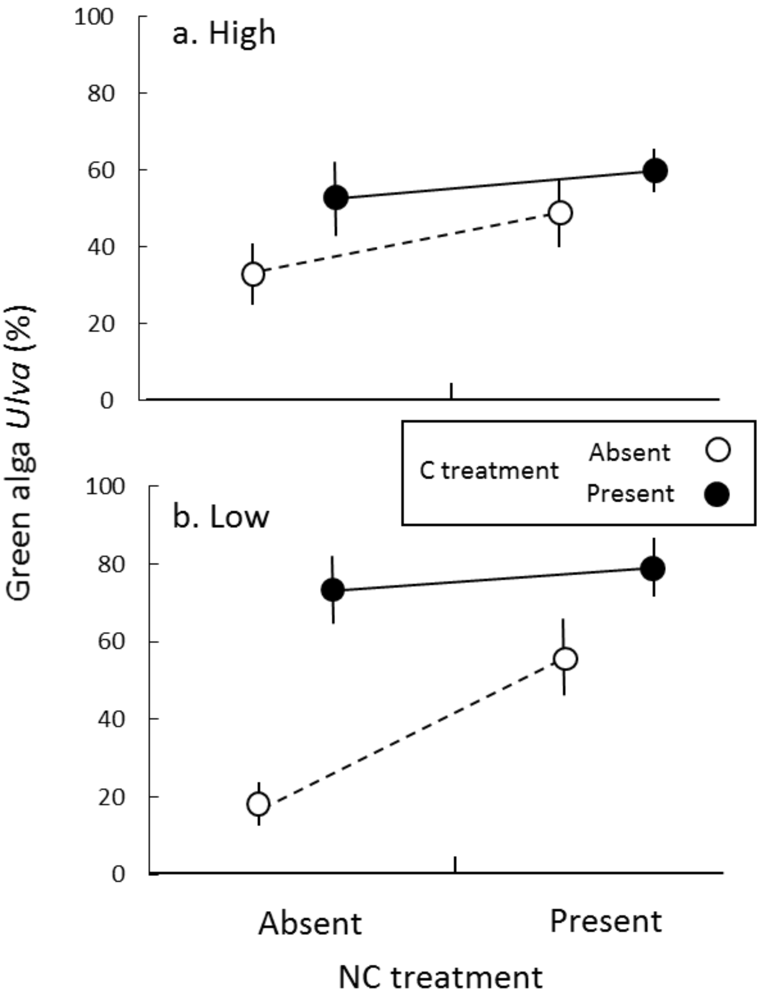

Fig. 3. Percentage of *Ulva* in the total algal coverage in the presence/absence of the consumptive (i.e., DMIIs) and non-consumptive treatment (TMIIs) in (a) high-density and (b) low-density plots. Responses are shown as the mean ± SE of four replicates within each treatment. C and NC represent the consumptive and non-consumptive treatments, respectively.

TABLES

Table 1. Results of ANOVA on the effects of consumptive and non-consumptive treatments and their interaction terms (a) on the limpet's traits (direct effects), and (b) on the total percent cover of algae and proportion of *Ulva* in the total algal coverage (indirect interactions) at high and low limpet densities. *P*-values below 0.05 are underlined.

(a)	Growth				Disappeared				Feeding rate			
	High		Low		High		Low		High		Low	
Source	$F_{1,7}$	P	$F_{1,7}$	P	$F_{1,7}$	P	$F_{1,7}$	P	$F_{1,7}$	P	$F_{1,7}$	P
CE	0.14	0.72	5.79	0.047	53.29	0.0002	0.56	0.48	0.46	0.52	12.58	0.009
NCE	0.15	0.27	3.03	0.13	0.52	0.49	0.41	0.54	0.36	0.57	6.75	0.04
Tidal level	0.01	0.91	1.31	0.29	3.36	0.11	1.72	0.23	1.30	0.29	0.01	0.92
CE × NCE	0.12	0.74	0.06	0.82	3.64	0.10	0.01	0.93	0.03	0.86	1.62	0.24
Tidal level × CE	0.08	0.79	0.55	0.48	3.90	0.09	0.56	0.48	0.10	0.76	2.24	0.18
Tidal level × NCE	2.28	0.17	0.31	0.60	0.75	0.41	2.83	0.14	2.31	0.17	0.02	0.89
Tidal level × CE × NCE	4.22	0.08	0.002	0.96	1.82	0.22	2.19	0.18	1.45	0.27	0.03	0.88
Population size	0.56	0.48	2.25	0.18	5.35	0.054	3.57	0.10	0.07	0.80	4.88	0.06

(b)	Total cover				Proportion of *Ulva*			
	High		Low		High		Low	
Source	$F_{1,7}$	P	$F_{1,7}$	P	$F_{1,7}$	P	$F_{1,7}$	P
DMII	0.01	0.91	0.81	0.40	1.68	0.24	20.39	0.003
TMII	0.02	0.90	0.18	0.69	1.99	0.21	8.08	0.03
Tidal level	0.27	0.64	0.20	0.67	4.47	0.07	2.34	0.17
DMII × TMII	0.61	0.46	0.46	0.97	0.05	0.83	2.63	0.15
Tidal level × DMII	3.77	0.09	0.08	0.79	1.48	0.26	1.68	0.24
Tidal level × TMII	0.91	0.37	0.51	0.50	2.41	0.16	3.07	0.12
Tidal level × DMII × TMII	0.34	0.58	0.003	0.96	1.82	0.22	0.94	0.36
Population size	0.23	0.65	0.10	0.76	0.82	0.39	0.62	0.46

Table 2. Sizes of CEs and NCEs on the growth rate of *S. sirius*, the percentage of individuals that disappeared, and the per capita feeding rate on *Ulva* sp., and sizes of DMIIs and TMIIs on the total algal cover and percent cover of the resource alga *Ulva* sp. *P*-values below 0.05 are underlined.

	Direct interactions, mean (SE)				Indirect interactions, mean (SE)	
	Growth rate	Disappeared	Feeding rate		Total cover	Proportion of *Ulva*
CE				DMII		
High densities	0.01 (0.08)	-0.49 (0.14)	-0.29 (0.10)	High densities	-0.004 (0.001)	0.33 (0.18)
Low densities	-0.36 (0.05)	-0.22 (0.40)	-0.70 (0.19)	Low densities	-0.005(0.001)	1.44 (0.08)
t (6)	-3.86	0.63	-1.96	*t* (6)	-0.43	5.65
P	0.01	0.55	0.10	*P*	0.68	0.001
NCE				TMII		
High densities	-0.06 (0.13)	0.76 (0.16)	-0.20 (0.16)	High densities	-0.003 (0.002)	0.31 (0.14)
Low densities	-0.35 (0.11)	0.63 (0.60)	-0.71 (0.13)	Low densities	-0.002 (0.004)	1.22 (0.33)
t (6)	-1.68	-0.21	-2.45	*t* (6)	0.41	2.58
P	0.14	0.84	0.049	*P*	0.69	0.04

Notes: Estimates of CE and DMII strengths are based on the consumptive treatment in the absence of non-consumptive treatment, and estimates of NCE and TMII strengths are based on the non-consumptive treatment in the absence of consumptive treatment.

Table 3. The principal results of the study.

	High density		Low density	
(a) Direct effects	CE	NCE	CE	NCE
Growth	0	0	−	0
Disappeared	−	0	0	0
Feeding rate	0	0	−	−
(b) Indirect interactions	DMII	TMII	DMII	TMII
Total cover	0	0	0	0
Proportion of *Ulva* sp.	0	0	+	+

Notes: Effects are consumptive or non-consumptive for direct effects, density-mediated, or trait-mediated for indirect interactions. Plus signs (+) indicate positive effects, minus signs (−) indicate negative effects, and zero (0) signs indicate the lack of significant effects.

APPENDIX DATA

Appendix figures

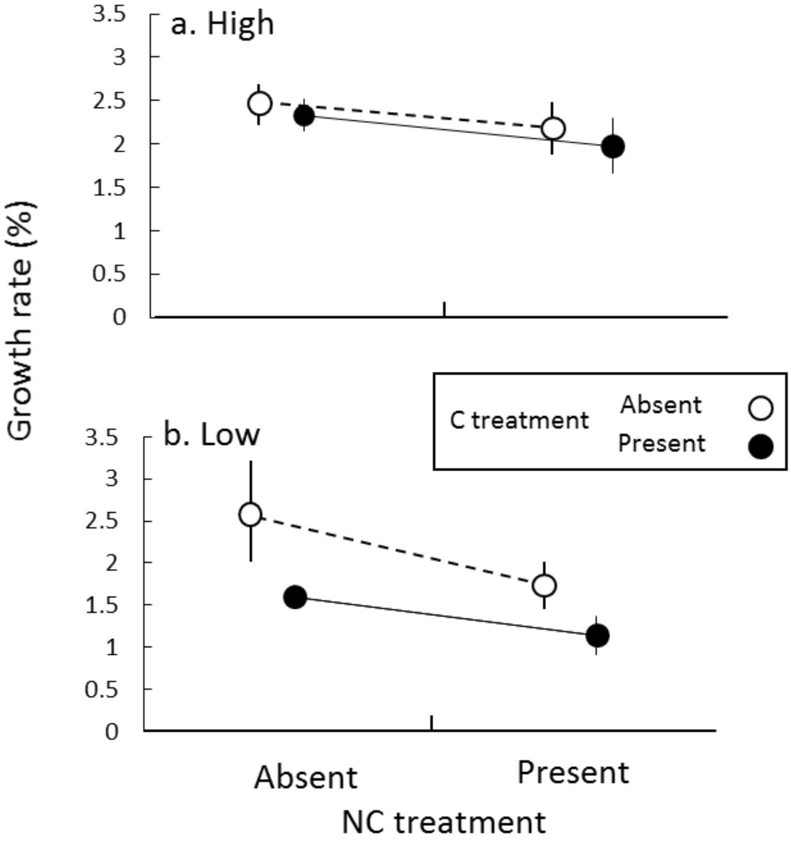

Fig. A1. Growth rate of limpets, *Siphonaria sirius*, over the 29-day experiment in the presence/absence of the consumptive and non-consumptive treatments in (a) high-density and (b) low-density plots. Responses are shown as the mean ± SE of four replicates within each treatment. C and NC represent the consumptive and non-consumptive treatments, respectively.

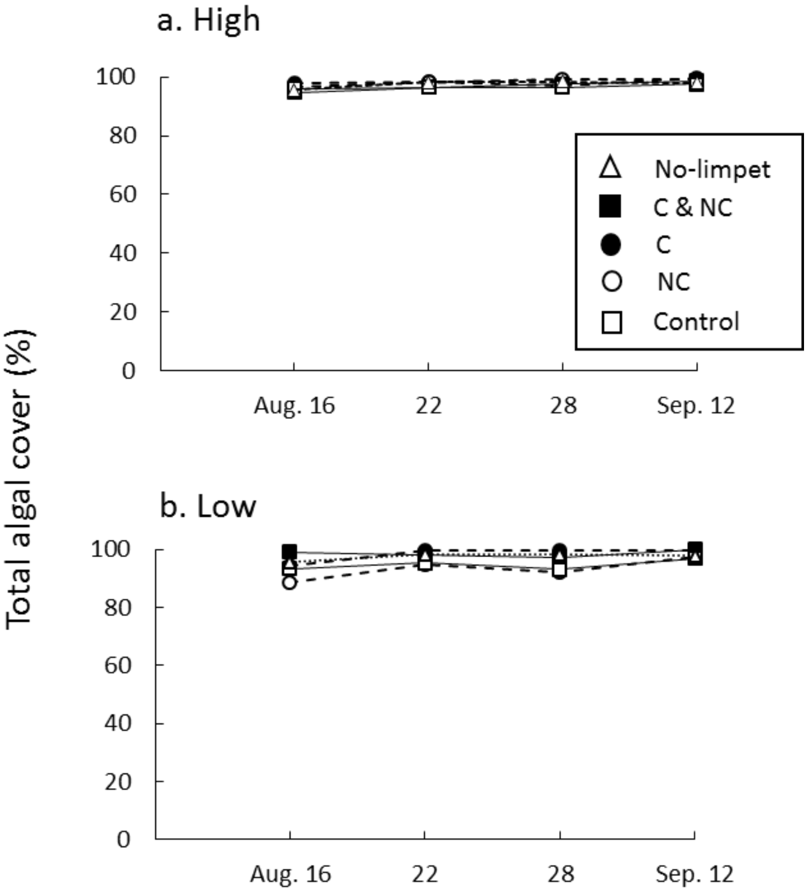

Fig. A2. Temporal changes in the total percent cover of algae (predominantly *Ulva* and *Lithoderma*) in (a) high-density and (b) low-density plots. C and NC represent the consumptive and non-consumptive treatments, respectively.

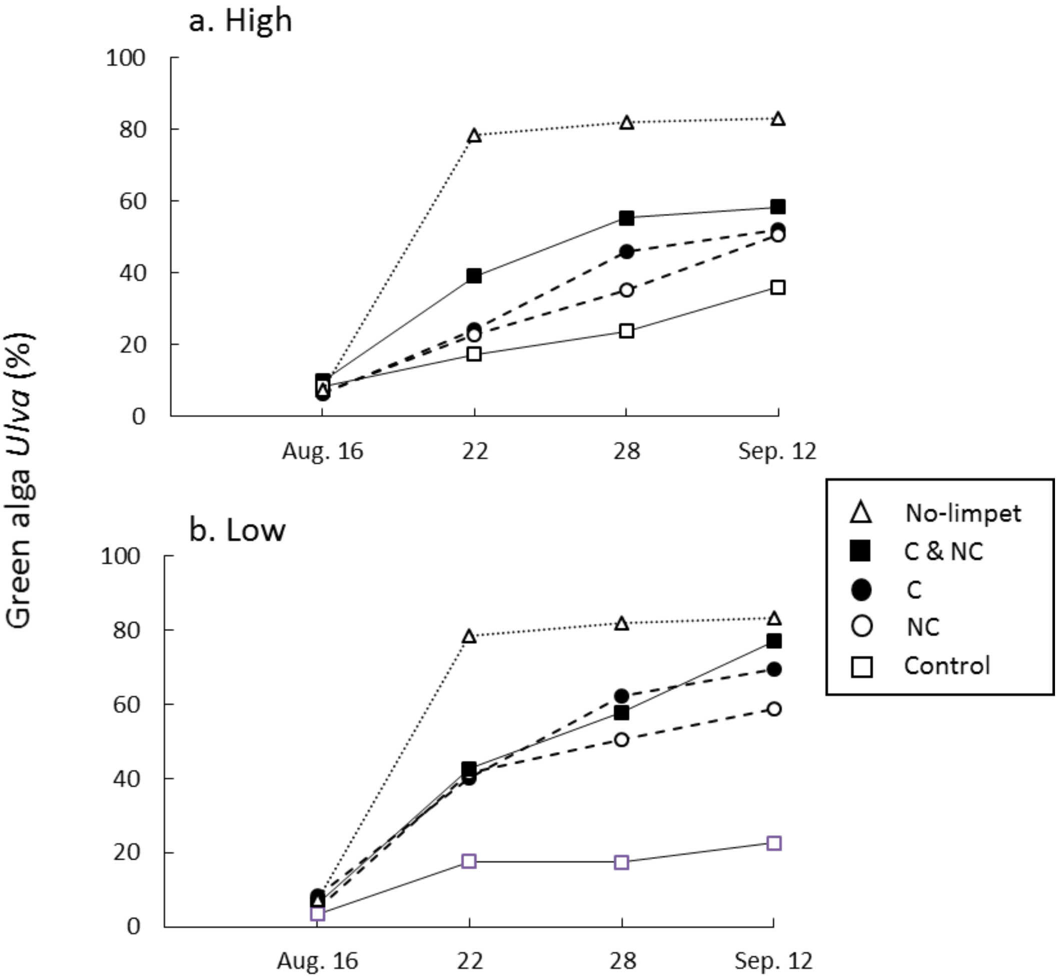

Fig. A3. Temporal changes in the percent cover of *Ulva* in the total algal coverage in (a) high-density and (b) low-density plots. C and NC represent the consumptive and non-consumptive treatments, respectively.

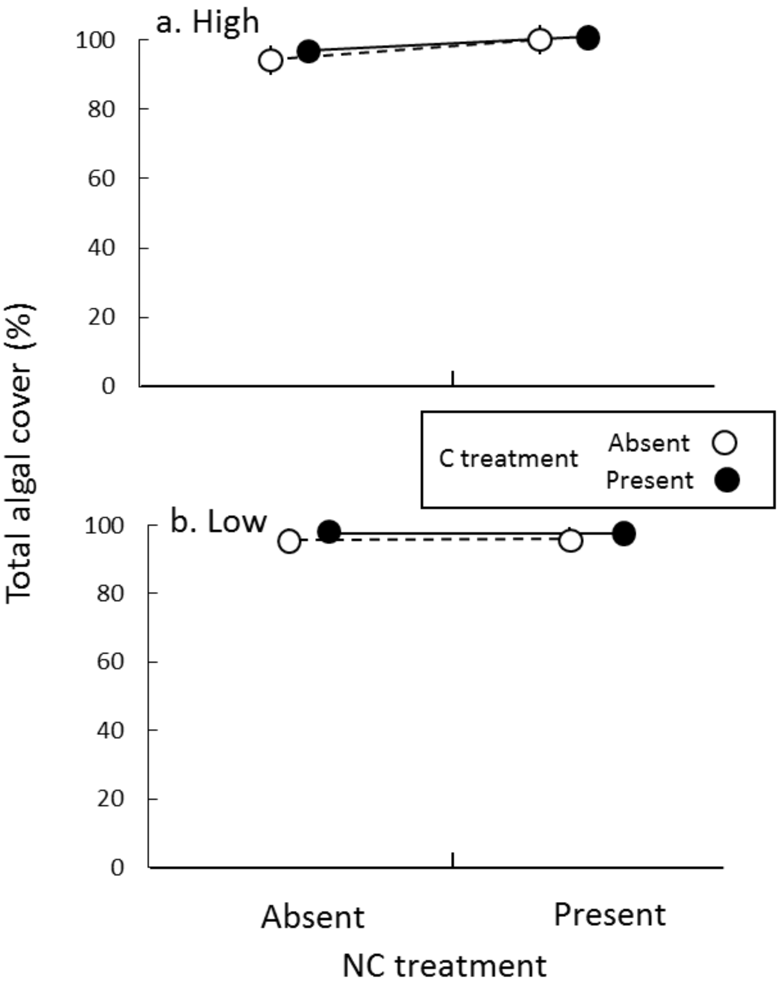

Fig. A4. Total percent cover of algae in the presence/absence of the consumptive (i.e., DMIIs) and non-consumptive treatment (TMIIs) in (a) high-density and (b) low-density plots at the end of the experiment. Responses are shown as the mean ± SE of four replicates within each treatment. C and NC represent the consumptive and non-consumptive treatments, respectively.

CHAPTER 4

Seasonal Dynamics of a Rocky Shore Ecosystem Driven by Fluctuating Indirect Interactions

Abstract. Accurately evaluating strengths of direct (i.e. consumptive and non-consumptive) effects and indirect (density- and trait-mediated) interactions is crucial to understand the mechanisms and dynamics of ecosystems. However, such evaluation has not been conducted *in situ* long enough to take seasonality and life cycle of community components into account. I conducted a 9-month (from summer to spring) field experiment in an intertidal rocky shore involving the carnivorous snail *Thais clavigera* (“C type” *sensu* Abe 1985), its prey, the limpet *Siphonaria sirius*, and the resources, the cyanobacterium (blue-green alga) *Lithoderma* sp. and the green algae *Ulva* spp. From summer to autumn, consumptive and non-consumptive effects of the predator had the opposite (positive and negative, respectively) effects on the prey. The predator affected not only adults but also new recruits of the prey. Both indirect interactions decreased the coverage of *Lithoderma* and increased *Ulva*, but trait-mediated interactions lasted longer than density-mediated interactions. Through winter to spring, predation pressure was low and no direct effects nor indirect interactions were detected. Investigations in seasons with low predation pressure highlight previously unnoticed time dependence of species interactions, such as offset of density-mediated interactions by density compensation processes of the prey and non-consumptive effects on the number and growth of new recruits.

INTRODUCTION

Ecosystems are one of the most complicated systems in nature, where organisms interact each other not only directly but also indirectly (Schmitz 2010). In food chains, indirect interactions of the predators are transmitted to the resources through one or more intervening species in two ways: (1) density-mediated indirect interactions (DMIIs) that are mediated by numerical responses of the prey (consumptive effects, CEs) and (2) trait-mediated indirect interactions (TMIIs) mediated by changes in the prey’s behavioral, morphological, physiological, and life history traits (non-consumptive effects, NCEs) (Abrams 1995, Lima 1998). Recently, many studies showed that the strengths of NCEs and TMIIs were similar or even greater than those of CEs and DMIIs (e.g. Werner and Peacor 2003), and NCEs and TMIIs are recognized as keys to understand a wide range of ecological issues such as population dynamics, community organization, ecosystem functions, and coevolution (Ohgushi et al. 2012).

Studies on DMIIs and TMIIs have revealed that the strengths of these indirect interactions are influenced by many characteristics of community components including foraging mode of predators (Schmitz 2010), prey’s habitat, density, and physiological conditions (Trussell et al. 2006, Alexander et al. 2013, Matassa and Trussell 2014, Gravem and Morgan 2015, Wada et al. 2015), and identity and quantity of resources

(Luttbeg et al. 2003, Trussell et al. 2008). However, most previous studies were conducted under spatially (e.g. in the laboratory or mesocosms) and/or temporally (from days to weeks) limited situations. This is problematic because the strengths of all the above-mentioned factors may show spatial and temporal variations, as shown for prey's antipredator behavior in response to variable predation risk (Matassa and Trussell 2011, 2014). In response, several studies aimed at quantifying indirect interactions in experiments with little or no limitation of space (Trussell et al. 2002, 2004, Takagi and Miyashita 2015, Wada et al. 2013, 2015).

Likewise, conclusions drawn from short-term experiments may not reflect long-term dynamics by two reasons. First, results from short-term experiment may mask effects of seasonality of each organism on direct effects and indirect interactions and on subsequent ecosystem dynamics (Schmitz 2000, Luttbeg et al. 2003). Second, short-term experiments may hinder detecting the different responses of prey at different life history stages (Ellrich et al. 2016). Seasonality and life history stages are important in intertidal ecosystems where many invertebrates have pelagic larvae and seasonal settlement patterns, which is a key potential determinant of community structure (Underwood and Fairweather 1989, Menge et al. 2010).

Although theoretical studies have suggested the importance of long-term study in evaluating DMIIs and TMIIs (McPeek and Peckarsky 1998, Luttbeg et al. 2003, Abrams 2008), empirical studies are still scarce (but see Takagi and Miyashita 2015). Previous long-term studies either focused on TMIIs only (Raimondi et al. 2000), did not distinguish between DMIIs and TMIIs (e.g. Paine 1966, Fletcher 1987, Wootton 1992 for intertidal rocky shore), or were conducted with spatial limitations (Hoverman and Relyea 2012, Manzur et al. 2014). Thus, no studies have evaluated the strengths of direct effects and indirect interactions in the field for long enough to take seasonality and life history of the prey into account.

I conducted a 9-month (July to April) field experiment for a trophic cascade in a lower intertidal rocky shore (Wada et al. 2013, 2015). I evaluated the CEs (by scheduled manual removal) and NCEs (by allowing emission of the odors from feeding predators) of a carnivore predator (the muricid snail *Thais clavigera* “C type” sensu Abe 1985) on its prey (the pulmonate limpet *Siphonaria sirius*). Moreover, I measured the indirect interactions of the predator on the resources (the cyanobacterium or the “blue-green alga” *Lithoderma* sp. and the green algae *Ulva* spp.). The snail *T. clavigera* is the major predator on *S. sirius*, and *S. sirius* is a dominant herbivore in the study area (Wada et al. 2013, 2015). The limpets have their own home scars and show homing behavior (Ohgushi et al. 1953), and the adults rarely emigrate from their home ranges even in the presence of their predator (Wada et al. 2013). They feeds much more on *Ulva* spp. than on *Lithoderma* sp., so the rock surfaces within their home ranges are covered with *Lithoderma* whereas *Ulva* grows outside the home ranges. My previous experiment (Wada et al. 2013) conducted for one month in summer showed that both DMIIs and TMIIs decreased the coverage of *Lithoderma* sp and increased *Ulva* sp. through competitive interactions between the algae. However, each component of the community has seasonality. *Thais clavigera* forages actively in summer and the number of individuals at lower intertidal zone increases because of migration from upper intertidal zones, but it reduces foraging activity and migrates upwards gradually towards winter (Abe 1989). The limpet *S. sirius* spawns in summer (Iwasaki 1995a) and its larval settlement occurs mainly in autumn and continues up to spring (Iwasaki 1993). *Ulva* increases in cover from late summer to winter (Iwasaki 1993) whereas *Lithoderma* shows the opposite trend (Wada et al. 2013).

The main purpose of this study was to evaluate temporal changes in the strengths of the indirect interactions (DMIIs and TMIIs) of the predator snail on the algal community structure, via the direct effects (CEs and NCEs) on the survival and the growth of the prey limpet. Settlement and post-settlement growth of newly recruited limpets were also examined. In particular, I first hypothesized that the strengths of the direct and indirect species interactions fluctuate seasonally because of the temporal changes in the predation pressure and seasonality of the prey and the algae. Second, NCEs may reduce not only the number of adult individuals through causing higher mortality (Wada et al. 2013) but also new recruits through settlement avoidance and/or high initial mortality, as known in barnacles (Ellrich et al. 2015). Third, reduction of prey density by predation may be soon compensated by density dependent processes of the prey such as higher growth and survival. Lastly, TMIIs may last longer than DMIIs because of the NCEs on the new recruits and the density compensation processes of the prey.

MATERIALS AND METHODS

Experimental treatments

I conducted a field experiment from 8 July 2013 to 1 April 2014 (268 days). Two treatments (consumptive and non-consumptive treatments) were applied to the limpets following standard experimental design (Peacor and Werner 2001, Griffin and Thaler 2006, Trussell et al. 2006), as in Wada et al. (2013, 2015). The presence and absence of each treatment were fully crossed and thus four treatment groups were made: consumptive only, non-consumptive only, both treatments, and neither treatment (control). In addition, I established “no-limpet” plots, where all *S. sirius* individuals were removed throughout the experiment, to observe any changes in the algal community in the absence of limpets. Moreover, to estimate the natural predation rate on *S. sirius*, I monitored unmanipulated rocks as “natural’’ plots. These six treatments were randomly assigned to 54 independent sandstone rocks (N = 9 replicates) in an intertidal rocky shore near Seto Marine Biological Laboratory, Wakayama, Japan (33.75° N, 135.28° E). The rocks were at least 0.8 m apart from each other in the lower intertidal area (at depths of from -86 to -36 cm relative to the mean tide level), and the uppermost side of each rock was used as the experimental plot. The size of the experimental plots (0.11 – 0.62 m^2) did not differ among the six treatment groups (likelihood $x^2_5 = 0.72$, $P = 0.98$, generalized linear model involved gamma distribution and ln-link function including the six treatment groups as a fixed factor). The plots were larger than the home ranges of the limpets (Wada et al. 2013). In addition, the edge of each plot, except for the natural plots, was lined (ca. 5 cm width) with a paint containing copper powder (Denka, Tokyo, Japan) to prevent migration of benthic animals including the limpets. The copper paint had no visible negative effects on the limpet’s survival, growth, or activity (Wada et al. 2013, 2015). I removed all herbivores (except for the limpets) and carnivores from the experimental plots at the beginning and during the experiment whenever found. Fifteen randomly selected limpets in each plot were individually marked with paint markers.

Census periods were taken 12 times, each lasting 4 – 10 days at the intervals of 12 – 30 days from July to early December, and then once in late January and once in March - April. A consumptive treatment was applied to estimate the strengths of CEs of the predator on the prey and resulting DMIIs. I removed a fixed proportion (0 – 13.0%) of both marked and unmarked limpets randomly with a scraper at each census. This proportion was based on the natural predation rate on limpets, estimated as the average mortality in the natural plots (with predators) minus the average mortality in the control plots (without predators) during the previous interval of the census. At the start of the experiment, I applied the predation rate measured in advance for two weeks in the same plots (7.8% per week per limpet).

A non-consumptive treatment was used to quantify the strengths of NCEs and TMIIs resulting from odors of feeding predators. For the non-consumption treatment, I attached a small meshed cylindrical cage (25 mm tall, 77 mm in diameter) using water-resistant epoxy glue (Konishi Corporation, Osaka, Japan) to the middle of the upper edge of each plot. I placed *T. clavigera* (shell height 16.5 – 24.1 mm) and *S. sirius* (individuals previously removed from the consumptive plots) into each cage within all plots receiving the non-consumptive treatment to permit the release of odors of the snails feeding on the limpets. The numbers of predator individuals (0 – 7) and the prey individuals (0 – 21) placed in each odor cage were determined by the mean number observed in the natural plots measured at each census and the natural predation rate (i.e., the same proportion as those removed in the consumptive treatment), respectively. The prey individuals were introduced into the odor cage after each census, and dead predators were replaced with new ones whenever found. I also attached the cages to the plots without non-consumptive treatments, but no predators or limpets were introduced.

Estimation of direct effects and indirect interactions

To estimate the CEs and NCEs on *S. sirius*, the survival and growth rates of adult individuals were measured. Moreover, to evaluate these effects on the next generation, the number of new recruits and their growth were also monitored. The survival of limpets was censused six times, on 8 July, 7 August, 17 September, 4 December 2013, 28 January, and 31 March 2014. The survival rate between two successive censuses was calculated as the number of marked individuals in the later census divided by the number in the earlier census. Manually removed individuals were excluded from the calculation. Individuals disappeared from the plots were regarded as being dead because the limpets seldom leave their home ranges (Wada et al. 2013) and I did not find marked limpets outside the plots. The growth of each marked limpet was evaluated by measuring shell lengths six times (on 8 July, 9 August, 18 September, 22 November 2013, 28 January, and 31 March 2014). The number of limpet recruits was counted in two quadrats (18.5 × 18.5 cm) placed just below and the right of the cage, and the data of two quadrats were summed. Moreover, the shell lengths of five randomly selected recruits were measured at each census in each plot. The census on the recruits started in November, when they grew to visually identifiable size (> 2.0 mm in shell length) and was conducted four times during the observation period (on 5 November, 6 December 2013, 1 February, and 1 April 2014).

To estimate the strengths of DMIIs and TMIIs on the algae, the coverage of *Lithoderma* sp. and *Ulva* spp. was measured. I took photographs of two quadrats put in the same places as the measurement of recruits of the limpet. Within each quadrat, I plotted 169 equidistant points on the photographs and counted the number of points that were superimposed on each algal species, and the values for the two quadrats were summed. The coverage of the algae was censused six times, on 11 July, 8 August, 19 September, 4 December 2013, 29 January, and 31 March 2014.

Statistical analyses

Survival rates of the limpets were analyzed using Cox proportional hazards regression model. I tested the effects of the presence/absence of the consumptive and non-consumptive treatments, and their interaction in the model. Analyses of direct effects on the limpets and indirect interactions on the algae were conducted using generalized linear mixed models (GLMMs). The analysis of size of marked limpets involved a gamma distribution (ln-link function), including the presence/absence of the consumptive and non-consumptive treatments, the date of census (as a categorical factor), and their interactions as fixed factors. Mixed models were necessary to account for the effects of identities of the marked individuals and the rocks. Analyses of the number and size of recruits involved Poisson and gamma distributions (ln-link function), respectively. The models included treatments, census date, and their interactions as fixed factors and rock identity as a random factor.

To evaluate the strengths of DMIIs and TMIIs on the algae, I used the number of points with *Lithoderma* sp. on the photographs. I used *Lithoderma* data because it grows within the home range of the limpet and therefore its response to the limpet' grazing is more direct than that of *Ulva*, but the major results did not change even if I used *Ulva* instead. The number of points with *Lithoderma* was analyzed with Poisson distribution (ln-link function), with the treatments, census date, and their interaction terms as fixed factors and rock identity as a random factor. The analyses were conducted twice, with and without incorporating the natural logarithm of the number of points with *Ulva* as a covariate. This is because *Lithoderma* and *Ulva* were major components in the algal community in this area with a strong competitive interaction (Wada et al. 2013, 2015). When applicable, *post hoc* tests were conducted among census dates of the same treatment or among treatments of the same date. All statistical analyses were performed with R (version 3.2.1; R Development Core Team 2015).

In all the analyses except for survival, the significance of main effects and interactions was determined with likelihood x^2 tests. To facilitate interpretation, I show results for particular factors adjusted for the effects of other components of statistical models (least-squares means; Milliken and Johnson 1984). For graphical purposes, I back-transformed results from the scale of the link function to the original scale of measurement, which results in asymmetrical standard errors.

RESULTS

Direct effects of snails on adult limpets

I found strong seasonality both in the number of the predator snails and the prey limpets. The number of *T. clavigera* (Fig. 1a) and their predation rate per *S. sirius* individual (Fig. 1b) both decreased from summer to autumn and remained almost zero in winter, when the snails migrate to upper tidal flats (Abe 1989). Then both the number and predation rate of *T. clavigera* increased slightly in spring. The number of the prey gradually decreased from summer to autumn, then rapidly increased due to new recruitment (Fig. 1c). Because of deaths of new recruits, the prey number slightly decreased in spring.

Survivorship of marked limpets (> 1 year cohorts) decreased throughout the experimental period, and it showed a greater decline from July to September than in the later seasons (Fig. 2a). In particular, the decline was drastic in plots receiving the non-consumptive treatment from August to September, leading to a significant NCEs between the two census dates ($b \pm \mathrm{SE} = -0.17 \pm 0.06$, $z = -2.71$, $P < 0.01$). In contrast, no effects of the consumptive treatment and the interaction with the non-consumptive treatment were detected between any of the two consecutive census dates (all $|z| < 1.3$, $P > 0.2$).

The shell length increased throughout the experimental period (Fig. 2b, c) and differed significantly among census dates (Table 1). In contrast, both the initial (CEs: $|z| = 0.15$, $P = 0.88$, NCEs: $|z| = 0.30$, $P = 0.77$) and the final shell lengths (CEs: $|z| = 0.18$, $P = 0.86$, NCEs: $|z| = 0.59$, $P = 0.56$) were similar irrespective of treatments (Fig. 2b, c). However, both treatments influenced shell length differently with time (see CE × Date and NCE × Date in Table 1). On one hand, CEs accelerated shell growth from August to November (Fig. 2b). This was shown by the significant shell growth without the consumptive treatment from July to August ($|z| = 3.11$, $P = 0.02$) but the lack of growth with the consumptive treatment ($|z| = 2.61$, $P = 0.10$) in the same period. Likewise, the shell length still increased without the consumptive treatment from November to March ($|z| = 5.55$, $P < 0.001$), but not with the consumptive treatment ($|z| = 1.38$, $P > 0.7$). On the other hand, NCEs decelerated the prey growth (Fig. 2c). In particular, shell length without the non-consumptive treatment was greater in August than in July ($|z| = 4.35$, $P < 0.001$), whereas no apparent growth was seen with the non-consumptive treatment in the same period ($|z| = 1.28$, $P > 0.79$). Furthermore, shell length increased from November to March with the non-consumptive treatment ($|z| = 3.95$, $P < 0.05$), but not in the absence of the non-consumptive treatment ($|z| = 2.73$, $P > 0.05$).

Direct effects on newly recruited limpets

In general, the number of newly recruited limpets (0 year cohort) increased up to February and then slightly declined irrespective of the treatments (Fig. 3a, b). As a result, the number of recruits differed among census dates (Table 2), although the final numbers in April were similar irrespective of the treatments (consumptive treatment: $|z| = 0.58$, $P = 0.56$, non-consumptive treatment: $|z| = 1.03$, $P = 0.30$). Both the consumptive and non-consumptive treatments influenced the number differently among census dates (CE × Date, NCE × Date; Table 2). The consumptive treatments accelerated the increment of recruits during autumn (Fig. 3a), resulting in a significant difference between November and December ($|z| = 5.22$, $P < 0.001$), but not in plots without the treatment ($|z| = 2.31$, $P > 0.05$). In contrast, the non-consumptive treatment decelerated the increment during the same period (Fig. 3b). Notably, the non-consumptive treatment reduced recruitments by 57.5% in November and 47.9% in December as compared with the cases lacking the treatment.

The shell lengths of recruits increased throughout the experimental period (Fig. 3c) and differed among census dates (Table 2). Although the final shell lengths of recruits in March attained similar sizes irrespective of treatments (*post hoc* tests between two treatment groups $|z| < 1.56$, $P > 0.40$), the growth patterns considerably differed, as shown in the significant interactions with census date (Table 2). Obviously, the shell lengths were small in plots with the non-consumptive treatment early in winter (NCE, CE × NCE, Fig. 3c). The shell length increased in plots with the non-consumptive treatment from January to April ($|z| = 6.93$, $P < 0.001$), but not in the other plots (all $|z| < 2.10$, $P > 0.18$). Thus, the non-consumptive treatment had a negative effect on the shell length of newly recruited limpets in the early recruitment season.

Indirect interactions on algae

The coverage of *Lithoderma* sp. differed among census dates (Table 3). The coverage was similar among the treatment groups at the start (all $|z| < 0.62$, $P > 0.93$) and the end (all $|z| < 1.40$, $P > 0.50$). However, the temporal changes considerably differed, as shown in the significant interactions with census dates (Table 3). First, the coverage of *Lithoderma* in the unmanipulated (control) plots was maintained comparatively well throughout the experimental period (all comparisons between two consecutive census dates were $|z| < 1.16$, $P > 0.79$, Fig. 4). Next, in plots with the DMIIs only, *Lithoderma* coverage decreased up to September, resulting in a significant difference between July and September ($|z| = 18.08$, $P < 0.001$). After that, it increased towards January, as evidenced by a significant difference between September and January ($|z| = 10.94$, $P < 0.001$), followed by a stationary period from January to March ($|z| = 0.70$, $P = 0.99$). The initial decrease in *Lithoderma* coverage was also observed in plots with the TMIIs only, from July to September ($|z| = 18.42$, $P < 0.001$). However, the decrease lasted up to December and then *Lithoderma* increased, making no difference between September and January ($|z| = 2.06$, $P = 0.37$). The increase lasted until March, resulting in a significant difference between January and March ($|z| = 5.58$, $P < 0.001$). Finally, the seasonal pattern in plots with both DMIIs and TMIIs were similar to those in plots with DMIIs only. The initial decrease, although stronger than in other plots, lasted only until September, resulting in a significant difference between July and September ($|z| = 27.87$, $P < 0.001$). *Lithoderma* increased from September to January ($|z| = 18.68$, $P < 0.001$), then became stationary from January to March ($|z| = 0.27$, $P = 1.00$). Taken together, both DMIIs and TMIIs decreased *Lithoderma* coverage in the early period of the experiment but later these effects were alleviated. The TMIIs toward *Lithoderma* lasted longer than the DMIIs.

The inclusion of the coverage of *Ulva* in the analysis ($b \pm \mathrm{SE} = -0.01 \pm 0.003$, $z = -5.20$, $P < 0.001$) did not influence the main results (Table 3). This indicates that the DMIIs and TMIIs toward *Lithoderma* coverage does not depend on the effects of *Ulva*.

DISCUSSION

The results in this long-term field experiment support my first prediction that the strengths of both direct effects and indirect interactions fluctuate seasonally (Fig. 5). In accordance with the variable intensity of predation pressure, both direct effects of the predator on the prey and indirect interactions of the predator on the algae were remarkable in summer, but they became weakened as time passed. Among indirect interactions, TMIIs lasted longer than DMIIs, supporting my fourth prediction.

However, neither direct effects nor indirect interactions were detected in spring. To understand the possible mechanisms, I first examine each component of direct effects in periods with high and low predation pressure, respectively. Then I discuss the implications of indirect interactions and the time dependent nature of these species interactions.

Direct effects in period with high predation pressure

The survival rate of marked limpets decreased in the presence of the non-consumptive treatment from August to September (Fig. 2a), when the predation rate was high (Fig. 1b). The result is consistent with the results in my previous study (Wada et al. 2013) conducted in the same season. The higher mortality could result from a reduced feeding rate and an increased energy loss due to escaping under the non-consumptive treatment (Walzar and Schansberger 2009, Wada et al. 2013). Moreover, the limpet *S. sirius* consumes energy during summer for copulation and spawning and for moving downwards to avoid desiccation (Iwasaki 1995a, b). Thus, a shift in the energy budget in response to NCEs appears to have resulted in the higher mortality.

Both the consumptive and the non-consumptive treatments changed the growth pattern of adult limpets, but the effects were the opposite (Fig. 2b, c). As in higher mortality, the slower growth under the non-consumptive treatment could be due to lower energy gain and higher energy loss (Wada et al. 2013). In contrast, growth was more rapid under the consumptive treatment likely due to decreased intraspecific competition (Trussell et al. 2006, Wada et al. 2015), which supports my third prediction. In addition, the increase in the preferred alga *Ulva* instead of *Lithoderma* by DMIIs (Fig. 4) might have further helped their rapid growth (plant-mediated bottom-up effect; Ohgushi et al. 2007).

Direct effects in period with low predation pressure

From November to April, when predation pressure was low, the consumptive and the non-consumptive treatments had the opposite effects also on limpet recruits. The consumptive treatment increased the number of recruits in autumn and winter, whereas the non-consumptive treatment reduced both the number and the shell lengths. The effects of the consumptive treatment can be explained by the reduced intraspecific competition due to simulated predation (Wada et al. 2015). The fewer recruits under the non-consumptive treatment may be due to the avoidance of the predator odor by the larvae, as known in crabs (Welch et al. 1997) and barnacles (Johnson and Strathmann 1989; Ellrich et al. 2015). However, higher mortality just after settlement cannot be excluded in this study because the limpets were counted after they reached 2 mm in shell length because of the difficulty in identification. Likewise, the small shell sizes of recruits under the non-consumptive treatment could result from either slower growth or delayed settlement in response to the predator odor.

Thus, the results show that predators affect not only adults, but also recruits of the prey, supporting my third prediction. Few studies have evaluated responses of prey at different life history stages (Ellrich et al. 2016). However, prey at different life history stages may show different responses to the predation risk and the supply of new recruits can be a critical determinant of community structure, especially in marine ecosystems (Underwood and Fairweather 1989, Menge et al. 2010).

However, the positive CEs and negative NCEs on adults and recruits diminished in the period, and the size of adult and the size and number of recruits were almost the same in all treatment groups by spring (Fig. 5). Moreover, the recruitment recovered limpet density. These have deep implications in considering indirect interactions.

Indirect interactions

Both DMIIs and TMIIs decreased *Lithoderma* and increased *Ulva* until September, with stronger effects when both were simultaneously applied (Fig. 4), as my previous studies demonstrated (Wada et al. 2013, 2015). A new and important finding in the present study is that TMIIs lasted longer than DMIIs and acted until December. This is understandable because the survival of adult individuals and the number and growth of new recruits of *S. sirius* were all lower under NCEs even in winter. In contrast, almost all components of CEs (recruitment, survival, and growth of limpets) showed negative density dependence. Therefore, simulated predation under the consumptive treatment was compensated by the increased number and size of the remaining and newly settled limpets, and these processes offset the DMIIs earlier than TMIIs.

Interestingly, in March - April, algal community structure as well as the limpet population were almost the same among plots irrespective of the treatment received. This result implies that the community structure was “reset” in spring. This reset means that the previous effects of predators on the community structure does not last one year, and therefore the 9-month experimental period is long enough to monitor the dynamics of the ecosystem. In a modeling study simulating a terrestrial ecosystem, Schmitz (2000) showed that the direct effects and indirect interactions of a predator (spiders) monitored for short term could predict longer-term dynamics by taking seasonality into consideration. In an empirical study with a deer-plant-butterfly system, Takagi and Miyashita (2015) demonstrated that the relative strength of DMIIs and TMIIs strongly depended on the observational period. However, the present study is the first, to my knowledge, to evaluate the strengths of direct effects and indirect interactions *in situ* for a long time to take into account the seasonality and life history differences of ecosystem components.

Implications for temporal dynamics of indirect interactions

Many studies have evaluated the strengths of direct effects and indirect interactions under high predation pressure (Ohgushi 2012). However, evaluating their strengths under low predation pressure is important because prey and resources will recover their populations in periods with low predation pressure by new recruitment and regeneration, as shown in the present study. This recovery process is crucial for the long-term maintenance of the community. Therefore, it is essential to evaluate the direct effects and indirect interactions in periods with both high and low predation pressure to understand the dynamics of ecosystems.

In summary, this 9-month field study revealed that 1) seasonality of the community components influenced the strengths of direct effects and indirect interactions; 2) direct effects acted both the adults and new recruits of the prey; 3) TMIIs lasted longer than DMIIs; but 4) community structure was reset in spring. However, this study does not cover a whole life span of the prey (presumably 2 - 3 years; Iwasaki 1993). Moreover, evaluating inter-annual fluctuations in the community structure may be important in marine ecosystems with highly variable settlement, mass mortality, and growth patterns (Underwood and Fairweather 1989, Connell et al. 1997, Uthicke et al. 2009). Therefore, even longer-term studies on the fluctuating direct effects and indirect interactions will greatly advance the understanding of mechanisms and dynamics of complicated ecosystems.

LITERATURE CITED

Abe, N. 1985. Two forms of *Thais clavigera* (Küster, 1858). Venus (the Japanese Journal of Malacology) 44:15-26.

Abe, N. 1989. Interactions between carnivorous gastropods and their sessile animal prey at a rocky intertidal shore. Physiology and Ecology Japan 26:1-38.

Abrams, P. A. 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. The American Naturalist 146:112-134.

Abrams, P. A. 2008. Measuring the population-level consequences of predator-induced prey movement. Evolutionary Ecology Research 10:333-350.

Alexander, M. E., J. T. A. Dick, and N. E. O’Connor. 2013. Trait-mediated indirect interactions in a marine intertidal system as quantified by functional responses. Oikos 122:1521-1531.

Connell, J. H., T. P. Hughes, and C. C. Wallace. 1997. A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. Ecological Monographs 67:461-488.

Ellrich, J. A., R. A. Scrosati, and M. Molis. 2015. Predator nonconsumptive effects on prey recruitment weaken with recruit density. Ecology 96:611-616.

Ellrich, J. A., R. A. Scrosati, C. Bertolini, and M. Molis. 2016. A predator has nonconsumptive effects on different life-history stages of a prey. Marine Biology 163:5.

Fletcher, W. J. 1987. Interactions among subtidal Australian sea urchins, gastropods, and algae: effects of experimental removals. Ecological Monographs 57:89-109.

Gravem, S. A., and S. G. Morgan. 2015. Prey state alters trait-mediated indirect interactions.in rocky tidepools. Functional Ecology, in press.

Griffin, C. A. M., and J. S. Thaler. 2006. Insect predators affect plant resistance via density- and trait-mediated indirect interactions. Ecology Letters 9:338-346.

Hoverman, J. T., and R. A. Relyea. 2012. The long-term impacts of predators on prey: inducible defenses, population dynamics, and indirect effects. Oikos 121:1219-1230.

Iwasaki, K. 1993. The role of individual variability in limpet resting site fidelity and competitive ability in the organization of a local rocky intertidal community.

Physiology and Ecology Japan 30:31-70.

Iwasaki, K. 1995a. Foraging and spawning rhythms of the pulmonate limpet *Siphonaria sirius* (Pilsbry): switching of activity period by a diurnal forager. Journal of Molluscan Studies 61:275-288.

Iwasaki, K. 1995b. Dominance order and resting site fidelity in the intertidal pulmonate limpet *Siphonaria sirius* (Pilsbry). Ecological Research 10:105-115.

Johnson, L. E., and R. R. Strathmann. 1989. Settling barnacle larvae avoid substrata previously occupied by a mobile predator. Journal of Experimental Marine Biology and Ecology 128:87-103.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions: What are the ecological effects of anti-predator decision-making? BioScience 48:25-34.

Luttbeg, B., L. Rowe, and M. Mangel. 2003. Prey state and experimental design affect relative size of trait- and density-mediated indirect effects. Ecology 84:1140-1150.

Manzur, T., F. Vidal, J. F. Pantoja, M. Fernández, and S. A. Navarrete. 2014. Behavioural and physiological responses of limpet prey to a seastar predator and their transmission to basal trophic levels. Journal of Animal Ecology 83:923–933.

Matassa, C. M., and G. C. Trussell. 2011. Landscape of fear influences the relative importance of consumptive and nonconsumptive predator effects. Ecology 92:2258-

2266.

Matassa, C. M., and G. C. Trussell. 2014. Prey state shapes the effects of temporal variation in predation risk. Proceedings of the Royal Society B 281:20141952.

McPeek, M. A., and B. L. Peckarsky. 1998. Life histories and the strengths of species interactions: combining mortality, growth, and fecundity effects. Ecology 79:867-879.

Menge, B. A., M. M. Foley1, J. Pamplin, G. Murphy, and C. Pennington. 2010. Supply-side ecology, barnacle recruitment, and rocky intertidal community dynamics: Do settlement surface and limpet disturbance matter? Journal of Experimental Marine Biology and Ecology 392:160–175.

Milliken, G. A., and D. E. Johnson. 1984. Analysis of messy data, Volume 1: Designed experiments. Van Nostrand Reinhold, New York.

Ohgushi, T., T. P. Craig, and P. W. Price. 2007. Ecological Communities: Plant Mediation in Indirect Interaction Webs. Cambridge University Press, Cambridge.

Ohgushi, T., O. J. Schmitz, and R.D. Holt. 2012. Trait-mediated indirect interactions. Cambridge University Press, Cambridge.

Ohgushi, R., T. Miura, and M. Kotani. 1953. Some observations on the homing behaviors of two species of limpets. Venus (Japanese Journal of Malacology) 17:219-

223.

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

Peacor, S. D., and E. E. Werner. 2001. The contribution of trait-mediated indirect effect to the net effects of a predator. Proceedings of the National Academy of Sciences of the United States of America 98:3904-3908.

Raimondi, P. T., S. E. Forde, L. F. Delph, and C. M. Lively. 2000. Processes structuring communities: evidence for trait-mediated indirect effects through induced polymorphisms. Oikos 91:353-361.

Schmitz, O. J. 2000. Combining field experiments and individual-based modeling to identify the dynamically relevant organizational scale in a field system. Oikos 89:471-484.

Schmitz, O. J. 2010. Resolving Ecosystem Complexity. Princeton University Press, Princeton, New Jersey, USA.

Takagi, S., and T. Miyashita. 2015. Time-scale dependency of host plant biomass- and trait-mediated indirect effects of deer herbivory on a swallowtail butterfly. Journal of Animal Ecology 84:1657-1665.

Trussell, G. C., P. J. Ewanchuk, and M. D. Bertness. 2002. Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. Ecology Letters 5:241-245.

Trussell, G. C., P. J. Ewanchuk, M. D. Bertness, and B. R. Silliman. 2004. Trophic cascades in rocky shore tide pools: distinguishing lethal and nonlethal effects. Oecologia 139:427-432.

Trussell, G. C., P. J. Ewanchuk, and C. M. Matassa. 2006. Habitat effects on the relative importance of trait- and density-mediated indirect interactions. Ecology Letters 9:1245-1252.

Trussell, G. C., P. J. Ewanchuk, and C. M. Matassa. 2008. Resource identity modifies the influence of predation risk on ecosystem function. Ecology 89:2798-2807.

Underwood, A. J., and P. G. Fairweather. 1989. Supply-side ecology and benthic marine assemblages. Trends in Ecology & Evolution 4:16-20.

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

Wada, Y., K. Iwasaki, and Y. Yusa. 2013. Changes in algal community structure via density- and trait-mediated indirect interactions in a marine ecosystem. Ecology 94:2567-2574.

Wada, Y., K. Iwasaki, and Y. Yusa. 2015. Prey density affects strengths of density- and trait-mediated indirect interactions of predators on an algal community. Journal of Experimental Marine Biology and Ecology 468:67-73.

Walzer, A., and P. Schausberger. 2009. Non-consumptive effects of predatory mites on thrips and its host plant. Oikos 118:934-940.

Welch, J. M., D. Rittschof, T. M. Bullock, and R. B. Forward. 1997. Effects of chemical cues on settlement behavior of blue crab *Callinectes sapidus* postlarvae. Marine Ecology Progress Series 154:143-153.

Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84:1083–1100.

Wootton, J. T. 1992. Indirect effects, prey susceptibility, and habitat selection: impacts of birds on limpets and algae. Ecology 73:981–991.

FIGURES

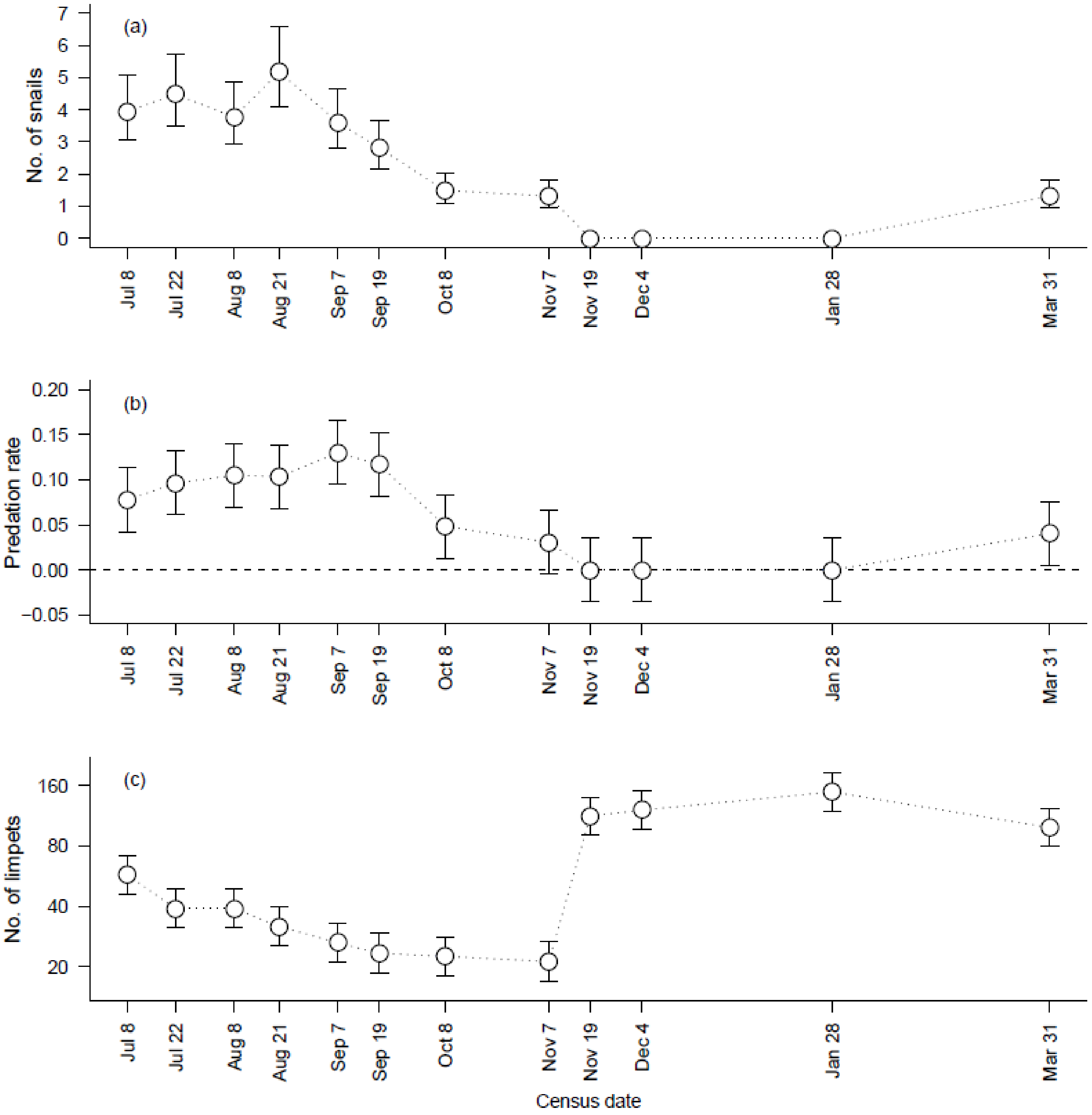

Fig. 1. Seasonal variations in least-squares mean (± SE) number of predators, *Thais clavigera* (a), their predation rate per limpet individual (b), and number of prey, *Siphonaria sirius* (c; shown in the logarithmic scale) in natural plots. In the panel (c), the number of newly recruited limpets in two quadrats in each plot was converted into the number in the plot to obtain the total number. All values were adjusted for effect of rock identity.

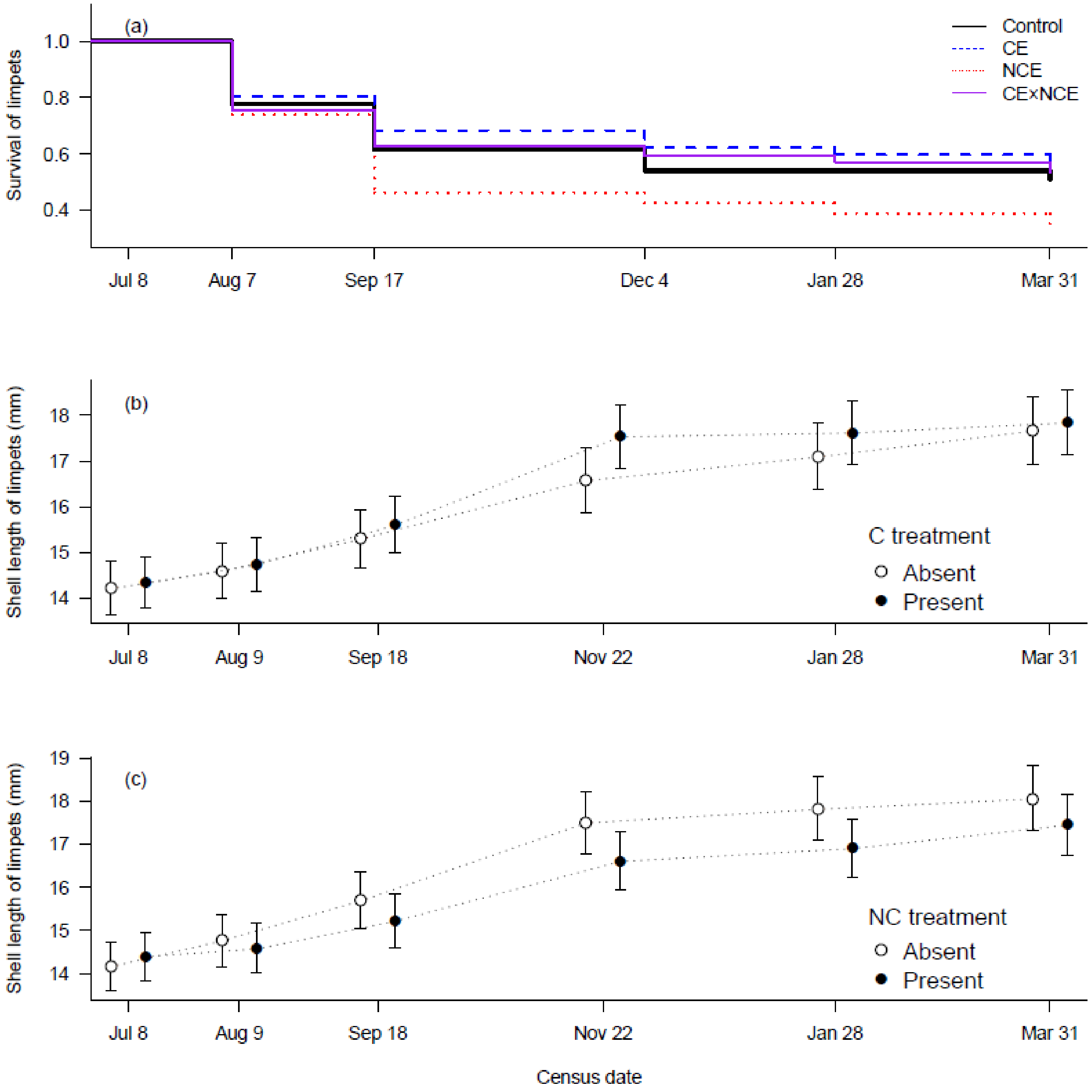

Fig. 2. Seasonal variations in survival rates (a) and least-squares mean (± SE) shell lengths of *Siphonaria sirius* limpets (b, c). The panels (b) and (c) show the effects of the presence/absence of the consumptive (C) and non-consumptive (NC) treatments, respectively. Shell length has been adjusted for the effects of non-consumptive and consumptive treatment, respectively, in (b) and (c).

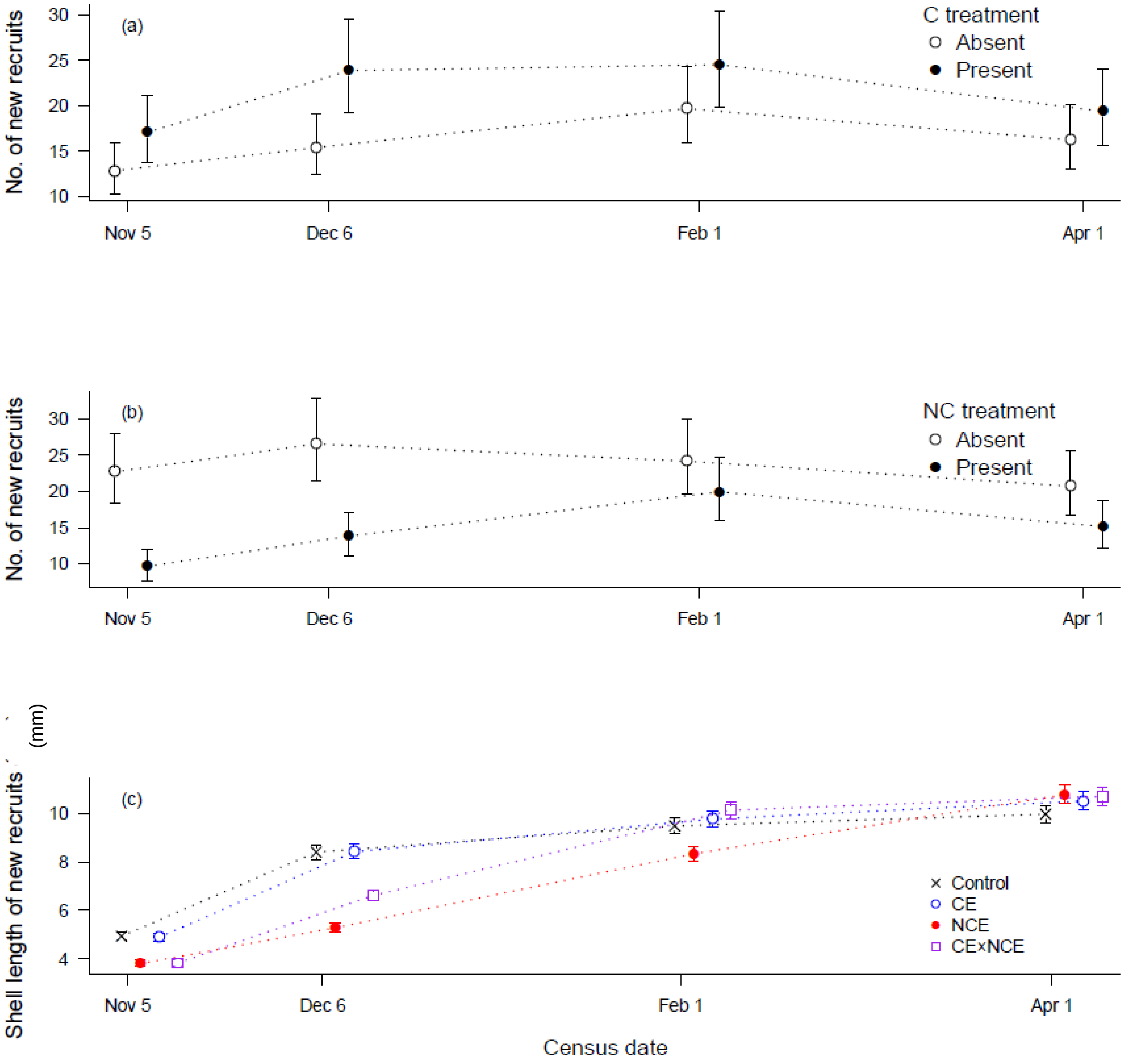

Fig. 3. Seasonal variations in least-squares mean (± SE) numbers (a, b) and shell lengths (c) of recruits of *Siphonaria sirius*. The panels (a) and (b) show the effects of the presence/absence of the consumptive (C) and non-consumptive (NC) treatments, respectively. Numbers of new recruits have been adjusted for the effects of non-consumptive and consumptive treatment, respectively, in (a) and (b).

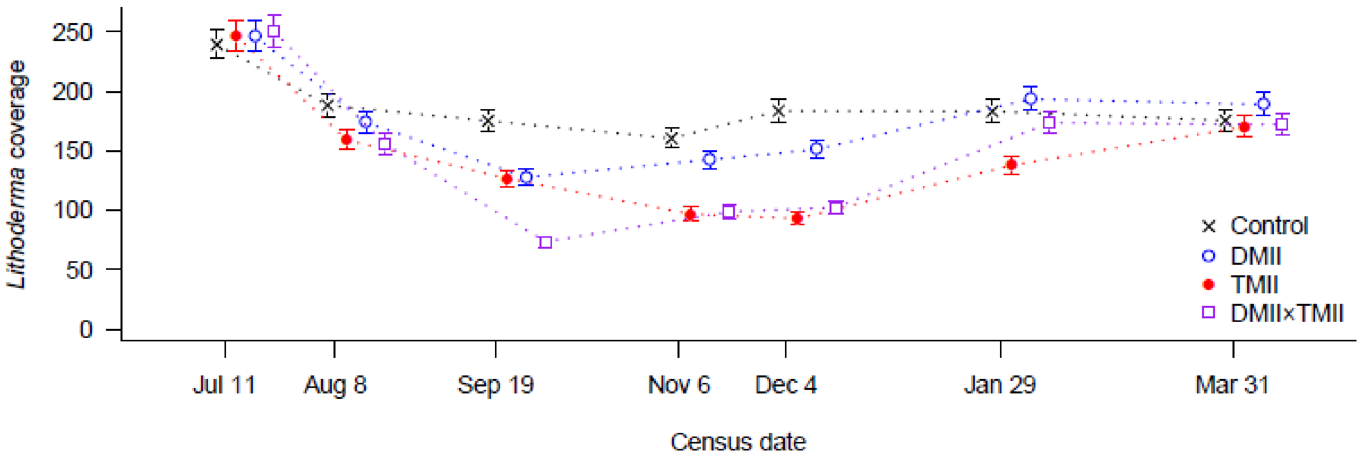

Fig. 4. Seasonal variations in least-squares mean (± SE) number of points with *Lithoderma* per 338 equidistant points on the photographs in the presence/absence of the consumptive (i.e. DMII) and non-consumptive treatment (i.e. TMII).

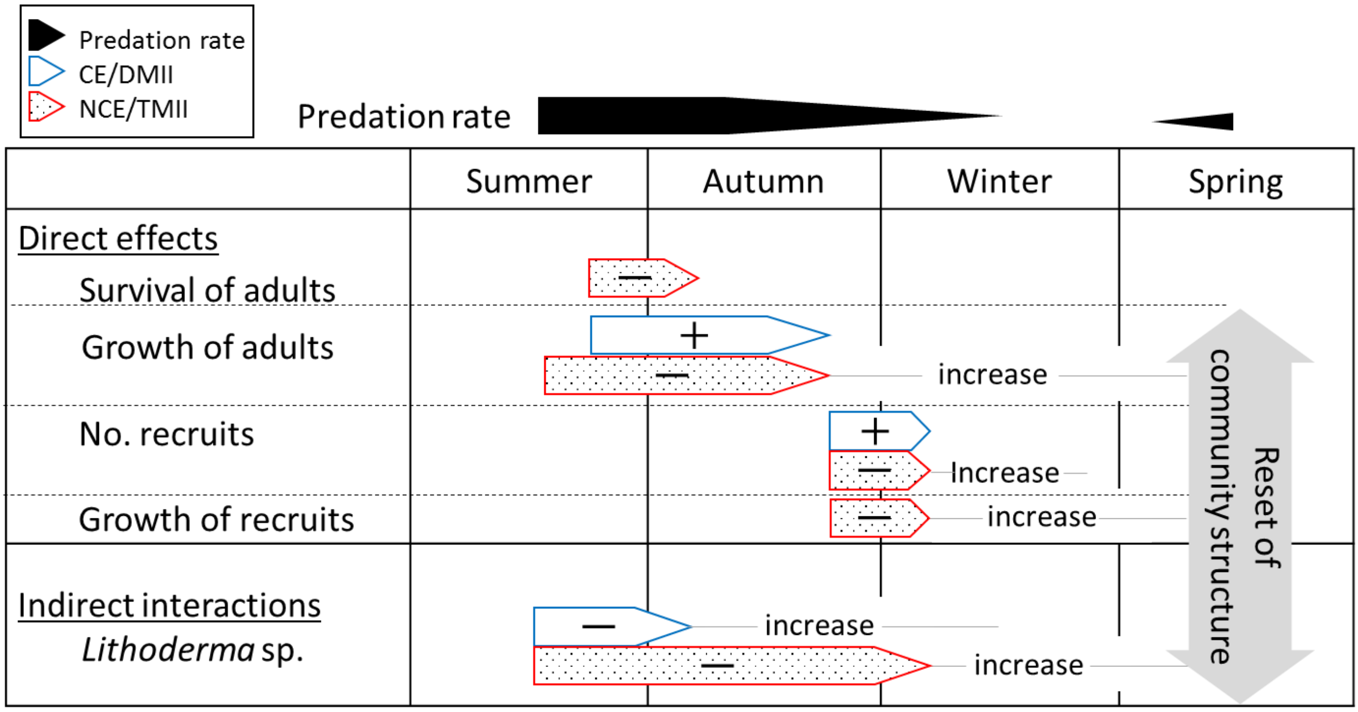

Fig. 5. Principal results of the study. Black, white, and dotted bars show predation rate per limpet, strengths of CEs and DMIIs, and strengths of NCEs and TMIIs, respectively. Plus signs (＋) and minus signs (－) indicate positive and negative effects, respectively.

TABLES

Table 1. Result of analysis of shell length of marked limpets by generalized linear mixed model that considered consumptive effects, non-consumptive effects, and their seasonal variation (census date).

Factor	Statistics
Consumptive Effect	$X^2_1 = 0.15$
Non-consumptive Effect	$X^2_1 = 0.24$
Census date	$X^2_5 = 1278.51^{***}$
CE × NCE	$X^2_1 = 1.30$
Date	$X^2_5 = 12.83^{*}$
NCE × Date	$X^2_5 = 28.53^{***}$
CE × NCE × Date	$X^2_5 = 9.91$

Notes: CE, NCE, and Date indicate Consumptive Effect, Non-consumptive Effect, and Census date respectively. *P*-values: $^{*}P < 0.05$; $^{***}P < 0.001$

Table 2. Result of analysis of number and shell length of newly recruited limpets by generalized linear mixed model that considered consumptive effects, non-consumptive effects, and their seasonal variation (census date).

Factor	Number	Shell length
Consumptive Effect	$X^2_1 = 0.88$	$X^2_1 = 5.20^{*}$
Non-consumptive Effect	$X^2_1 = 2.87$	$X^2_1 = 30.54^{***}$
Census date	$X^2_3 = 68.16^{***}$	$X^2_3 = 2735.41^{***}$
CE × NCE	$X^2_1 = 0.008$	$X^2_1 = 2.55$
CE × Date	$X^2_3 = 8.82^{*}$	$X^2_3 = 15.25^{**}$
NCE × Date	$X^2_3 = 58.91^{***}$	$X^2_3 = 147.71^{***}$
CE × NCE × Date	$X^2_3 = 5.15$	$X^2_3 = 18.65^{***}$

Notes: CE, NCE, and Date indicate Consumptive Effect, Non-consumptive Effect, and Census date, respectively. *P*-values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Table 3. Result of analysis of coverage of *Lithoderma* sp. by generalized linear mixed model that considered density- and trait-mediated indirect interactions, and their seasonal variation (census date). The analyses were conducted twice with or without logarithm number of *Ulva* sp.

Factor	without *Ulva* model	with *Ulva* model
Density-mediated indirect interaction	$X^2{}_1 = 1.44$	$X^2{}_1 = 1.06$
Trait-mediated indirect interaction	$X^2{}_1 = 25.54^{***}$	$X^2{}_1 = 23.57^{***}$
Census date	$X^2{}_5 = 2072.58^{***}$	$X^2{}_5 = 981.80^{***}$
DMII × TMII	$X^2{}_1 = 0.17$	$X^2{}_1 = 0.08$
DMII × Date	$X^2{}_5 = 227.16^{***}$	$X^2{}_5 = 225.68^{***}$
TMII × Date	$X^2{}_5 = 328.33^{***}$	$X^2{}_5 = 298.05^{***}$
DMII × TMII × Date	$X^2{}_5 = 49.61^{***}$	$X^2{}_5 = 46.78^{***}$
Ln(*Ulva* number)		$X^2{}_1 = 27.07^{***}$

Notes: DMII, TMII, and Date indicate Density- and Trait-mediated indirect interaction, and Census date, respectively. *P*-values: $^{***}P < 0.001$

CHAPTER 5

General Discussion

I conducted three experiments to evaluate the strengths of direct effects and indirect interactions *in situ* in a marine food chain involving the snail *Thais clavigera*, the limpet *Siphonaria sirius*, and the algae *Lithoderma* sp. and *Ulva* sp. The factors affecting them and annual dynamics were also investigated.

In Chapter 2, I conducted a 38-day field experiment from summer to autumn in 2010, and investigated how algal community changed in a rocky shore via density- and trait-mediated indirect interactions. The results showed that the strengths of non-consumptive effects (NCEs) were larger than those of consumptive effects (CEs). Moreover, the strengths of density-mediated indirect interactions (DMIIs) and trait-mediated indirect interactions (TMIIs) were of similar magnitudes and both brought similar changes in algal community (from *Lithoderma* to *Ulva*). Although the importance of TMIIs has been emphasized in recent studies conducted in the laboratory or field enclosures, TMIIs were not stronger than DMIIs when measured under natural conditions. It is important to evaluate indirect interactions of predators on resource community structure because changes in the community structure can give strong impacts on all organisms that utilize them (Ohgushi et al. 2012).

In Chapter 3, I conducted a 27-day field experiment from summer to autumn in 2011, and investigated how the algal community changes through DMIIs and TMIIs at different (high and low) densities of the prey. As a result, *Lithoderma* decreased and *Ulva* increased by both DMIIs and TMIIs only at low densities of the prey, whereas neither interactions were detected at high densities. The result shows that the prey's density is important in determining the strengths of TMIIs and DMII and resource community structure. It is necessary to focus on the characteristics of the prey, as well as those of the predators and the resources, because the prey are the key players to convey the indirect interactions from the predators to the resources and their roles may differ under various environmental conditions (Matassa and Trussell 2014).

In Chapter 4, I conducted a 265-day field experiment from the summer of 2013 to the spring of 2014 to evaluate seasonal changes in the intensities of direct effects and indirect interactions. Both direct effects and indirect interactions were stronger in summer, but they became weaker in winter in accordance with the seasonal change of predation pressure. Moreover, TMIIs lasted longer than DMIIs, but neither direct effects nor indirect interactions were detected in spring. This is the first study to reveal the long-term dynamics of an ecosystem *in situ* for a period long enough to incorporate seasonality and life history differences of the components. I suggest that studying such seasonal dynamics is important because of the time dependent nature of species interactions, such as offset of density-mediated interactions due to the negative density dependence of the prey and non-consumptive effects on the number and growth of new recruits.

Recent studies on indirect interactions have focused on the roles of indirect interactions in understanding mechanisms for the maintenance of communities (Ohgushi et al. 2012). However, most experiments were conducted under spatially and/or temporally limited situations, and four major problems existed due to such limitations (Chapter 1). First, the lack of *in situ* measurements of direct effects and indirect interactions were resolved by conducting a series of field experiments (Chapters 2 – 4). Second, the biased evaluation of top-down indirect interactions by measuring only the amount of resources was reconsidered by observing algal community structure (Chapters 2 – 4). Third, the roles of the prey’s characteristics in determining the strengths of the indirect interactions which had been neglected in the previous studies were revealed by focusing on the density of the prey (Chapter 3). Fourth, to deal with the shortcomings with short-term previous experiments, I conducted a 9-month field experiment and revealed the seasonal dynamics of the community driven by fluctuating indirect interactions (Chapter 4).

However, these studies are not long enough to cover a whole life span of the prey (presumably 2-3 years; Iwasaki 1993) and do not cover inter-annual fluctuations in the community structure. Therefore, as the next step, it is necessary to conduct an even longer study to cover the life span of the components and inter-annual dynamics, especially in marine ecosystems, where the components often show highly fluctuating densities due to simultaneous settlement, mass mortality, or catastrophic events (Connell et al. 1997, Uthicke et al. 2009).

LITERATURE CITED

Connell, J. H., T. P. Hughes, and C. C. Wallace. 1997. A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. Ecological Monographs 67:461-488.

Iwasaki, K. 1993. The role of individual variability in limpet resting site fidelity and competitive ability in the organization of a local rocky intertidal community. Physiology and Ecology Japan 30: 31-70.

Matassa, C. M., and G. C. Trussell. 2014. Prey state shapes the effects of temporal variation in predation risk. Proceedings of the Royal Society B 281: 20141952.

Ohgushi, T., O. J. Schmitz, and R. D. Holt. 2012. Trait-mediated indirect interactions. Cambridge University Press, Cambridge.

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

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LIST OF PUBLICATIONS

Wada, Y., K. Iwasaki, and Y. Yusa. 2013. Changes in algal community structure via density- and trait-mediated indirect interactions in a marine ecosystem. Ecology 94:2567-2574. (CHAPTER 2)

Wada, Y., Y. Miura, Y. Fujiwara, T. Yamamoto, K. Okoshi, and Y. Yusa. 2013. The first finding of the neustonic barnacle *Lepas pectinata* and nudibranch *Fiona pinnata* in the deep sea. Cahiers de Biologie Marine 54:437-440.

Matsuda, S., Y. Wada, and Y. Kitagishi. 2013. Roles of PI3K/AKT/PTEN pathway as a target for pharmaceutical therapy. The Open Medicinal Chemistry Journal 7:23-29.

Nakanishi, A., Y. Wada, Y. Kitagishi, and S. Matsuda. Link between PI3K/AKT/PTEN Pathway and NOX Protein in Diseases. *Aging and Disease* 5:pp203-211. 2014.

Matsuda, S., A. Nakanishi, A. Minami, Y. Wada, and Y. Kitagishi. Functions and characteristics of PINK1 and Parkin in cancer. *Frontiers in Bioscience* 20:pp491-501.

Wada, Y., K. Iwasaki, and Y. Yusa. 2015. Prey density affects strengths of density- and trait-mediated indirect interactions of predators on an algal community. Journal of Experimental Marine Biology and Ecology 468:67-73. (CHAPTER 3)

Akita, S., Y. Wada, K. Wada, and H. Torii. 2015. Variation and social influence of

bowing behavior by sika deer (*Cervus nippon*). Journal of Ethology 34:89–96.