BEHAVIOURAL ECOLOGY

Danielle M. Warfe · Leon A. Barmuta

Habitat structural complexity mediates the foraging success of multiple predator species

Abstract We investigated the role of freshwater macrophytes as refuge by testing the hypothesis that predators capture fewer prey in more dense and structurally complex habitats. We also tested the hypothesis that habitat structure not only affects the prey-capture success of a single predator in isolation, but also the effectiveness of two predators combined, particularly if it mediates interactions between the predators. We conducted a fully crossed four-factorial laboratory experiment using artificial plants to determine the separate quantitative (density) and qualitative (shape) components of macrophyte structure on the prey-capture success of a predatory damselfly, *Ischnura heterosticta tasmanica*, and the southern pygmy perch, *Nannoperca australis*. Contrary to our expectations, macrophyte density had no effect on the prey-capture success of either predator, but both predators were significantly less effective in the structurally complex *Myriophyllum* analogue than in the structurally simpler *Triglochin* and *Eleocharis* analogues. Furthermore, the greater structural complexity of *Myriophyllum* amplified the impact of the negative interaction between the predators on prey numbers; the habitat use by damselfly larvae in response to the presence of southern pygmy perch meant they captured less prey in *Myriophyllum*. These results demonstrate habitat structure can influence multiple predator effects, and support the mechanism of increased prey refuge in more structurally complex macrophytes.

D. M. Warfe · L. A. Barmuta
School of Zoology and Tasmanian Aquaculture and Fisheries Institute, University of Tasmania,
GPO Box 252-05 Hobart, Tasmania, 7001, Australia

Present address:
D. M. Warfe (✉)
Water Resources Division, Department of Primary Industries, Water and Environment,
13 St. John's Avenue,
New Town, Tasmania, 7008, Australia
e-mail: Danielle.Warfe@dpiwe.tas.gov.au
Fax: +61-3-62336881

Introduction

"Habitat structure" refers to the physical structures in space which support plant and animal communities (McCoy and Bell 1991). In vegetated aquatic systems such as lakes, lowland rivers, estuaries and marine littoral zones, habitat structure is provided by vascular macrophytes and macroalgae (Heck and Crowder 1991). In lowland rivers in particular, macrophyte beds can form an important link between the main channel and floodplain food webs, and at a larger scale, between riverine and terrestrial food webs (Davies and Humphries 1996; Dettmers et al. 2001). The importance of macrophytes as habitat in aquatic systems is demonstrated by the diverse and abundant communities they support, often many magnitudes greater than unvegetated areas (Crowder et al. 1998), and macroinvertebrate abundance and diversity commonly increase with increasing vegetation biomass or density (Heck and Wetstone 1977; Stoner and Lewis 1985; Dean and Connell 1987; Carlisle and Hawkins 1998). Patterns of macroinvertebrate abundance on different species of macrophytes are less clear, although it has been suggested macrophytes of a more complex morphology, with more finely divided leaf structure, should support a greater abundance and diversity of macroinvertebrates (Heck and Orth 1980; Rooke 1986). Macrophytes provide more food resources, because there is more space available for food attachment and collection, and more refuges from predation than in unvegetated areas (Crowder et al. 1998; Diehl and Kornijow 1998).

While fish predators have been shown to alter macroinvertebrate community composition in vegetated systems (Heck and Crowder 1991; Crowder et al. 1998), their effectiveness at capturing prey commonly declines as the amount of macrophyte structure increases (Coen et al. 1981; Heck and Thoman 1981; Savino and Stein 1982; Nelson and Bonsdorff 1990; Bettoli et al. 1992). These

studies illustrate the effects of macrophyte density, but ignore differences in shape. Some research has used differently shaped macrophyte species, showing predator success can vary between species, but only at one density (Coull and Wells 1983; Leber 1985; Persson and Eklov 1995), thus confounding the quantitative and qualitative components of habitat structure. To properly understand the role of habitat structure in mediating trophic interactions, the effects of the quantitative and qualitative aspects of structure need to be separated (McCoy and Bell 1991; Beck 2000).

Predator effectiveness will further depend on the behaviour of both predator and prey, and also on the presence of more than one predator—a situation more likely in natural circumstances. Prey are usually at risk from more than one predator at any one time, and, given the variability present in predator behaviours and prey responses, the effects of predators in isolation may not give an accurate picture of the overall effects of predation on prey (Soluk 1993; Sih et al. 1998). Investigations of multiple predators have shown that their impacts may be non-additive; i.e. their combined impact may not necessarily be obtained by simply summing the impacts of individual predators in isolation (Losey and Denno 1988; Soluk and Collins 1988; Martin et al. 1989; Soluk 1993; Morin 1995). Two types of non-additive effects have been documented: positive and negative. Negative non-additivity occurs when fewer prey are eaten by multiple predators than would be expected by adding together their individual predator impacts (Soluk and Collins 1988). This implies a reduction in predation risk for the prey due to negative interactions between the predators (Sih et al. 1998), and includes intraguild predation where one predator is not only a competitor but also a prey item of another predator (Polis 1989). Positive non-additivity occurs where more prey are consumed by both predators in combination than would be expected from their separate effects, and usually indicates facilitation, where one predator increases the vulnerability of prey to another predator (Losey and Denno 1988; Soluk and Collins 1988). These facilitative interactions often result from prey behaviour; in their response to one predator, prey can become more vulnerable to predation by a second, usually differently foraging, predator and therefore their risk of predation is greater in the presence of multiple predators (Soluk 1993; Sih et al. 1998; Swisher et al. 1998).

If habitat structure can influence predator–prey interactions, then it may also influence predator–predator interactions and hence the combined impacts of multiple predators. In the only published study to have investigated the effects of habitat structure on multiple predators, Swisher et al. (1998) showed the combined impact of bluegill sunfish (*Lepomis macrochirus*) and dragonfly larvae (*Erythemis simplicicollis*) was greater at low macrophyte densities, thereby exceeding additivity because prey were more easily detected by bluegills as they escaped dragonflies. As the density of macrophytes increased, bluegills were less able to detect the prey and the combined impact of both predators became additive (Swisher et al. 1998). At the time of writing, there were no published studies investigating the effects of both macrophyte density and shape on the impact of multiple predators.

This experiment was motivated from observations of macrophyte beds in a lowland river, the Macquarie River, in Tasmania, Australia. These beds are structurally diverse and have a diverse community of macroinvertebrates, of which a coenagrionid damselfly, *Ischnura heterosticta tasmanica*, is an abundant invertebrate predator (Davies and Humphries 1996; D. M. Warfe, unpublished data). The macrophyte beds also support large populations of the southern pygmy perch, *Nannoperca australis*, a small native fish which feeds on epiphytic macroinvertebrates (Humphries 1995; Warfe 2003). While neither damselflies nor pygmy perch are the top predators of the system (introduced brown trout, *Salmo trutta*, and redfin perch, *Perca fluviatilis*, are also present), they are potential competitors, sharing the same habitat and food resources, and intraguild predators such as pygmy perch also prey on damselflies (D. M. Warfe, personal observation; Humphries 1995). If macrophyte density can decrease a predator's foraging success (Crowder and Cooper 1982; Swisher et al. 1998), then it is also reasonable to predict a reduction in predator success as macrophyte shape becomes more structurally complex. Furthermore, if habitat structure can mediate pair wise predator–prey interactions, then it is reasonable to expect it to influence the combined impacts of multiple predators (Sih et al. 1998). This experiment investigated these expectations by testing the following hypothesis: that macrophyte shape and density are separate components of habitat structure and have potentially independent effects on the individual and combined impacts of two predator species, a coenagrionid damselfly and the southern pygmy perch. Specifically, we hypothesised that: (1) the predators, both individually and in combination, would consume more prey at low macrophyte densities regardless of macrophyte shape, (2) the predators, both individually and in combination, would consume more prey in the simplest macrophyte shape regardless of macrophyte density; and (3) the impact of both predators in combination would be greatest at lower macrophyte densities and in the simplest shape.

Materials and methods

Study animals

The species used in this experiment were collected from macrophyte beds in the Macquarie River (147°28′E, 41°57′S), a slow-flowing lowland river in the midlands of Tasmania, Australia. The southern pygmy perch, *N. australis*, is found throughout southeastern Australia in lakes, shallow wetlands and lowland rivers where it occurs in patches of dense macrophyte growth (Humphries 1995). Individuals reach up to 80 mm long and consume macroinvertebrates associated with macrophytes, such as amphipods, ostracods, chironomids, other dipteran larvae and mayflies (Humphries 1995). Pygmy perch were collected by backpack electrofishing (model 12-B POW, Smith-Root) and sweep-netting in the macrophyte beds. Fifty-eight pygmy perch were collected and held in one of two 60-l

aquaria with plenty of cover. A single fish was randomly selected and allocated to each pygmy perch treatment, and transferred to the second aquarium upon completion of the treatment combination. After all fish had been used once (and as each replicate required 5 days to complete), the selection procedure was repeated ensuring no individual fish was used more than twice in any one replicate, nor more than 5 times over the course of the experiment, nor on consecutive days. The average length (±1 SEM) of pygmy perch used in the experiment was 37.46±5.65 mm, and a positively significant body length:weight regression ($F_{1,26}$=363.34, $P<0.001$, r^2=0.93) estimated the average wet biomass of fish used was 1.37 g. Visual analysis of residuals versus fish identity indicated there were no anomalies arising from fish identity and that all fish displayed the same patterns of consumption.

The second predator was the coenagrionid damselfly, *I. heterosticta tasmanica*, which is a common member of the macrophyte-associated community (Davies and Humphries 1996; Humphries 1996). Preliminary field sampling showed it to be the most numerous invertebrate predator in the macrophyte beds of the Macquarie River, and gut analyses revealed a diet of predominantly chironomids, chydorids, and mayflies (D. M. Warfe, unpublished data). Five hundred and fifty damselflies were collected by sweep-netting in macrophytes and, like the pygmy perch, each individual was used more than once but not consecutively (final instar larvae were not used). Ten individuals were used in each damselfly treatment. Damselflies were sorted into sizes before each replicate was run and randomly picked from each size group so that the ten individuals used in any one treatment combination covered the range of damselfly sizes (9–18 mm length, 2.2–4.3 mm head width). Each group of ten damselflies had an average body length (±1 SEM) of 13.94±3.36 mm, average head width of 2.15±0.43 mm, and total wet weight of approximately 0.5 g.

Pygmy perch and damselflies were held in laboratory aquaria with plenty of natural macrophytes under a regime of 12 h light:12 h dark, at water temperatures of 13–15°C (which represented median water temperatures in the Macquarie River, Humphries 1995), and the experimental aquaria were kept under the same conditions throughout the experiment. Macroinvertebrates were collected to supplement a live food supply of *Daphnia* spp. and mosquito larvae for both predators. Mosquito larvae (*Anopheles* sp.) were used as prey because both predators readily consumed them and they were representative of the mobile prey common in the diets of these predators. All prey were collected from the Macquarie River and local ponds.

Experimental Design

The experiment comprised a fully crossed four-factorial design, replicated 7 times, where the factors were pygmy perch (present and absent), damselflies (present and absent), macrophyte density (five levels) and macrophyte shape (three levels).

The densities of macrophyte analogues used in the experiment (Table 1) were based on surveyed densities of natural macrophytes in the Macquarie River (D. M. Warfe, unpublished data), and the highest experimental density corresponded to the highest natural density observed in the field. Artificial imitations of three macrophytes, varying in shape and common to the Macquarie River (Humphries et al. 1996), were constructed to allow quantifiable differences in density to be achieved and to control against influences of secondary chemicals and autogenic change in the plants. Wooden dowling (9.6 mm diameter, 280 mm height) was used to represent the macrophyte of low structural complexity, *Eleocharis sphacelata*, which has a simple, cylindrical reed structure. Commercially produced plastic aquarium plants (280 mm height) were used to represent the macrophyte shapes of intermediate and high structural complexity, and have been specifically designed to resemble the structure of natural macrophytes as closely as possible (Tetra Secondnature, Blacksburg, Va., personal communication). The *Triglochin procera* analogue was a tufted plant with 18 strap-like leaves of varying lengths and

represented the intermediate level of habitat structural complexity. The *Myriophyllum variifolium* analogue had four stems of whorled, highly dissected leaves and represented the plant of the greatest structural complexity (Warfe 2003). The density of plant analogues was designated by the number of stems for *Eleocharis* and *Myriophyllum*, and the number of tufts for *Triglochin*, which may lead to a perceived underestimation of density for this latter plant. Each *Triglochin* tuft had 18 leaves of varying lengths, thus the overall number of structural components was roughly similar to that provided by the other two plants.

Each treatment combination was randomly allocated to separate opaque polyvinyl chloride tanks (350×200×280 mm height, 15 l), each equipped with a polystyrene base (to hold the plant analogues) and an air stone. Plants were added to each tank according to macrophyte shape and density treatments. The tanks were then filled with a mixture of tap water and fresh river water (collected from local streams), and 25 mosquito larvae were introduced into each tank. Pilot trials showed that this was more prey than the predators could eat during the experimental period. Fifteen minutes later, damselflies were added to their respective tanks, and 15 min after that, the pygmy perch were added. Twenty-four hours after the introduction of the fish, the predators were removed, the plants were removed and rinsed (collecting any stray damselflies and mosquito larvae), and the remaining mosquito larvae were counted. We also recorded qualitative observations of mosquito, damselfly and pygmy perch behaviour from both experimental and holding tanks during the experiment.

Data analysis

A fully crossed four-factorial ANOVA with five planned comparisons was carried out on the number of prey consumed. No transformation of the data was necessary because plots of residuals and normal probability showed no violations of the assumptions of the ANOVA. Planned linear and quadratic contrasts were carried out across the five levels of macrophyte density. Planned comparisons were also conducted on macrophyte shape: *Eleocharis* was contrasted with *Triglochin* and *Myriophyllum*, and *Triglochin* was contrasted with *Myriophyllum*. Simple effects tests were conducted for any significant interactions.

To test whether the number of prey consumed by both predators combined was additive, the amount consumed by each predator alone was incorporated into an additive-consumption model developed by Soluk (1993):

$$C_{pd} = N_{prey}(P_p + P_d - P_p P_d).$$

where C_{pd} is the predicted combined consumption for the initial prey density (N_{prey}), and P_p and P_d are the probabilities of prey being consumed by pygmy perch or damselflies, respectively, over a 24-h period. This model takes into account that the predicted

Table 1 Densities of each macrophyte analogue used in the experiment, expressed as the density of stems (d) per tank (d/tank) and per square metre (d/m^2)

Macrophyte analogue	*Eleocharis sphacelata*		*Triglochin procera*[a]		*Myriophyllum variifolium*	
Density level	d/tank	d/m^2	d/tank	d/m^2	d/tank	d/m^2
1	0	0	0	0	0	0
2	13	186	1.5	22	11	157
3	39	557	4.5	64	33	472
4	65	929	7.5	107	55	786
5	91	1300	10.5	150	77	1100

[a]*T. procera* was quantified by tufts rather than stems, and each tuft comprised 18 leaves of varying lengths (see text).

combined consumption cannot exceed the initial prey density. The predicted combined consumption values were compared to the observed combined consumption values using a paired t-test for each macrophyte shape (Swisher et al. 1998). Significance levels were not adjusted as macrophyte shape was considered to be independent. A significant difference between the predicted and observed values indicated a non-additive effect of the two predators when they were both present. All analyses were carried out using SYSTAT version 9 (Wilkinson 1999).

Results

Behavioural observations

Mosquito larvae appeared to move near and remain around the macrophyte analogues, regardless of their shape, although they were never seen inside the highly dissected leaf whorls of *Myriophyllum*. They displayed the same behaviour upon contact with either predator, swimming away rapidly, although prey capture by both damselflies and pygmy perch was usually successful at the first attempt.

Damselflies used macrophyte structure as a perch from which to capture the prey, but used each shape differently. In the structurally simple reed-like macrophyte, *Eleocharis*, they perched on the side of a stem and moved around to the other side if a pygmy perch was nearby. Likewise, on *Triglochin* (the tufted analogue of intermediate structural complexity) they perched on one side of a leaf, moving to the other side, or occasionally another leaf, on sighting the pygmy perch. On the highly dissected *Myriophyllum* analogue, damselflies would perch on the outside of the leaflets to capture prey, but would move to the inside, next to the main stem, if a pygmy perch was near. They were rarely observed to move back to the outside of the leaflets during the trials.

Pygmy perch also appeared to use the macrophyte structure, generally remaining close to the plants except to dart out and capture prey. In the trials with high macrophyte density they were difficult to see, but were observed to swim around more within areas of thick macrophyte density in the holding tanks.

Statistical analyses

Macrophyte density had no significant effect on the number of mosquito larvae consumed (Table 2); 45%±3.8 (mean±SE) of them were consumed at each density level.

Macrophyte shape significantly affected the number of prey consumed (Table 2). Planned comparisons showed that 50% of the prey were consumed in the *Eleocharis* analogue while 45% were consumed in the *Triglochin* analogue ($F_{1,300}=3.559$, $P>0.060$). Forty-one percent of the prey were consumed in the most structurally complex *Myriophyllum* analogue, which was significantly fewer than in *Eleocharis* ($F_{1,300}=10.530$, $P<0.001$), but not in *Triglochin* ($F_{1,300}=1.845$, $P>0.175$).

Table 2 Summary of four-way ANOVA results for the effects of shape, density, pygmy perch and damselflies on the number of prey consumed

Source of variation	df	SS	F-ratio	P
Shape[a]	2	0.448	5.311	0.005**
Density[b]	4	0.176	1.046	0.383
Pygmy perch[c]	1	19.712	467.733	0.000**
Damselflies[d]	1	6.042	143.377	0.000**
Shape×density	8	0.214	0.635	0.748
Shape×pygmy perch	2	0.128	1.515	0.222
Shape×damselflies	2	0.046	0.548	0.578
Density×pygmy perch	4	0.078	0.464	0.762
Density×damselflies	4	0.212	1.256	0.287
Pygmy perch×damselflies	1	1.764	41.856	0.000**
Shape×density×pygmy perch	8	0.263	0.781	0.620
Shape×density×damselflies	8	0.477	1.416	0.189
Shape×pygmy perch×damselflies	2	0.081	0.966	0.382
Density×pygmy perch×damselflies	4	0.064	0.380	0.823
Shape×density×pygymy perch×damselflies	8	0.315	0.935	0.488
Residual	300	12.643		

**$P<0.01$
[a]Shape refers to the three levels of macrophyte structural complexity: *Eleocharis*, *Triglochin* and *Myriophyllum* analogues
[b]Density refers to the five levels of macrophyte density (see Table 1)
[c]Pygmy perch refers to the presence or absence of pygmy perch predators
[d]Damselflies refers to the presence or absence of coenagrionid damselfly larvae

Both the damselfly and pygmy perch treatments were significant; however, there was also a significant interaction between the two factors (Table 2). Simple effects tests showed that in the absence of pygmy perch, damselfly larvae consumed 40% of the prey ($F_{1,150}=425.139$, $P<0.001$); they consumed significantly fewer prey (only 12%) in the presence of pygmy perch ($F_{1,150}=9.468$, $P<0.002$). Only 25 of a total possible 900 damselflies were not accounted for upon completion of the treatments where both predators were present, and were presumed to have been consumed by pygmy perch. When both predators were absent, 1±0.3% of prey were missing which indicated the significant differences observed in the other predator treatments were due to predator consumption rather than missing larvae.

The number of prey consumed by both predators combined was 10% less than that predicted by the additive-consumption model ($t_{104}=2.044$, $P=0.043$), which indicated negative non-additivity. Separate t-tests for each macrophyte shape showed that this negative non-additivity only occurred in the *Myriophyllum* analogue ($t_{34}=2.207$, $P=0.034$). The combined consumption of both predators was additive in both the *Triglochin* ($t_{34}=0.467$, $P=0.643$) and *Eleocharis* analogues ($t_{34}=0.739$, $P=0.465$).

Discussion

This experiment tested two separate components of habitat structure—macrophyte density and macrophyte shape—on the foraging success of two freshwater predators. As predicted, these components had separate and independent effects on predators; macrophyte density had no effect on the number of prey eaten by either predator, whereas macrophyte shape not only influenced the number of prey consumed by each predator alone, but also their combined impact. These results support the arguments proffered by McCoy and Bell (1991) and Beck (2000), that the independent effects of shape and density must be separated in order to gain a more thorough understanding of how trophic interactions are mediated by the environment in which they occur.

Macrophyte density

Contrary to our expectations, macrophyte density did not affect the number of prey captured by either predator, regardless of macrophyte shape. Most studies that have tested the influence of habitat structure on predator success have measured it as macrophyte density, and have found that predator success declines as density increases (Nelson 1979; Folsom and Collins 1984; Gilinsky 1984; Gotceitas and Colgan 1989; Lipcius et al. 1998; Swisher et al. 1998). A common explanation is that predator mobility is impeded by the structure (Diehl 1988; Heck and Crowder 1991), so pygmy perch may simply be too small to be physically hampered by macrophytes, as has been suggested for the similarly sized pinfish, *Lagodon rhomboides* (Stoner 1982).

There is evidence that predators may shift their mode of foraging as the habitat structure becomes more dense (Savino and Stein 1989). James (1994) found that the lined seahorse (*Hippocampus erectus*) shifted from a searching mode of foraging to an ambush strategy as the seagrass habitat became more dense. At low macrophyte densities, pygmy perch adopted a sit-and-wait strategy, remaining motionless except to dart out and capture prey. This may be due to their risk of predation by piscivorous fish such as brown trout and redfin perch, both present in the Macquarie River, and such trade-offs between predation risk and foraging have been documented for other small fish (Crowder and Cooper 1982; Werner et al. 1983; Persson and Eklov 1995; Jacobsen et al. 1997). Pygmy perch are too small to be hampered by the habitat structure and adopted a searching strategy at higher macrophyte densities, moving amongst plant stems and consuming prey as they encountered them. By changing their foraging strategy with habitat structure, pygmy perch can consume a similar amount of prey regardless of macrophyte density.

However, damselflies were also unaffected by macrophyte density suggesting that prey behaviour contributed to this pattern. Numerous studies have shown that prey can alter their behaviour depending on their risk of predation (Werner et al. 1983; McIntosh and Peckarsky 1996;

Beckerman et al. 1997), and mosquito larvae have been shown to select habitats on the basis of macrophyte density and shape, occurring at greater abundances in dense *Myriophyllum* (Orr and Resh 1991, 1992). Our results suggest that while mosquito larvae may be able to perceive higher macrophyte density as a better refuge from predation, they may move around more within that habitat, negating the effect of refuge so their risk of predation was unaltered as density increased. Had we used epiphytic prey rather than a constant number of swimming prey, increasing macrophyte density would increase the surface area available for predators to search, and our results may have been quite different.

Macrophyte shape

Macrophyte shape affected the ability of both damselfly larvae and pygmy perch to find and capture prey; significantly fewer prey were consumed in the most structurally complex *Myriophyllum* analogue than in the structurally simple *Eleocharis* analogue. Differently shaped macrophyte species can support different macro-invertebrate assemblages (Stoner and Lewis 1985; Cyr and Downing 1988; Chilton 1990; Humphries et al. 1996), which, given that predator efficiency can be lower in more complex structures, may be partly due to differential effects on predator success (Coull and Wells 1983; Leber 1985; Diehl 1988; Diehl and Kornijow 1998). In one of the few studies to investigate the separate effects of macrophyte shape and density on the foraging efficiency of fish predators, Dionne and Folt (1991) found that shape had far more effect on prey capture rates of pumpkinseed sunfish, *L. gibbosus*, than density. Prey can be more readily detected in macrophytes with simple leaves (or no leaves), thus more complex plants act as a prey refuge by making it more difficult for predators to locate prey and hence easier for prey to avoid capture (Heck and Orth 1980; Main 1987; Ryer 1988; Dionne and Folt 1991). This would explain why damselfly larvae also consumed fewer prey in *Myriophyllum*, because prey were harder to detect amongst the highly dissected leaves. In an experiment using *Elodea*, *Ceratophyllum* and *Myriophyllum*, Walsh (1995) also found that damselfly larvae were less effective at capturing swimming prey in *Myriophyllum*. The highly dissected leaves of *Myriophyllum* appear to make prey detection more difficult for visually feeding damselfly and pygmy perch predators, thereby providing an effective prey refuge.

However, plants considered structurally complex due to a fine-leaf structure can actually prove less effective as a prey refuge because large and broad leaves can allow prey to hide more effectively from predators (Edgar 1983). Stoner (1982) found that while fewer prey were captured by pinfish (*L. rhomboides*) with increasing seagrass density, prey were more readily detected and captured in the fine leafy seagrass species than the simple wide-bladed forms. Clearly, the morphology of different macrophyte species can have significant effects on the ability of

predators to find and capture prey, and hence on their refuge value to prey, but these effects are likely to depend to some degree on predator and prey behaviour.

Multiple predator effects

Facilitative interactions between multiple predators seem to be more common than negative interactions in the literature, and tend to occur because the avoidance behaviour displayed by a prey species to a predator species makes it more vulnerable to another predator species (Losey and Denno 1988; Martin et al. 1989; Soluk and Richardson 1997; Sih et al. 1998; Swisher et al. 1998). Negatively non-additive interactions can occur when there is interference between multiple predators (Soluk and Collins 1988; Soluk 1993), or when a prey's avoidance behaviour makes it less vulnerable to both predators, thus precluding any direct interference between them (Crowder et al. 1997).

Our hypothesis that habitat structure would not only influence the effects of each predator in isolation but also their combined impact was supported—the amount of prey consumed depended on the presence of a second predator species. However, like the effects of habitat structure on the foraging success of individual predators, the success of both predators combined was not mediated by macrophyte density, but by macrophyte shape alone. Pygmy perch and damselflies had an additive impact in the macrophyte analogues of low and intermediate structural complexity (*Eleocharis* and *Triglochin*), but fewer prey were consumed than expected in the most structurally complex *Myriophyllum* analogue. Damselfly larvae consumed 28% fewer prey in the presence of pygmy perch, indicating the non-additivity observed was due to a negative interaction between the predators.

Pygmy perch are not only competitors of damselfly larvae, but also intraguild predators (sensu Polis 1989) in that they also prey on damselflies (D. M. Warfe, personal observation; Humphries 1995). One reason explaining the negatively non-additive effects of two predators is that one predator reduces the abundance of the other (Morin 1995). Less than 3% of damselflies were never recovered from the treatments with both predators, and were presumed consumed by pygmy perch. While the number of damselflies eaten would be unlikely to have a noticeable impact on overall prey consumption, it does show they are at risk from predation by pygmy perch and may therefore possess some predator avoidance behaviour. Thus the negative non-additivity displayed by these predators may have arisen through behaviour modification rather than direct consumption.

Many odonates modify their behaviour accordingly in the presence of predators (Pierce 1988; McPeek and Peckarsky 1998). Damselflies have been shown to exhibit predator avoidance behaviour by hiding behind stems (Heads 1985), reducing their movements in the presence of fish predators (Koperski 1997), and even being able to assess the relative risk of predation conferred by different macrophyte species and modify their behaviour accordingly (Dionne et al. 1990). In the *Eleocharis* and *Triglochin* analogues, damselflies perched on a stem or leaf (respectively) would move to the other side when a pygmy perch was nearby, thus they were hidden but still able to capture prey. In *Myriophyllum*, however, damselflies perched on the outside of the leaflets would move to the inside, next to the main stem, and therefore were unable to capture prey swimming past. Thus their predator avoidance strategy reduced their ability to capture prey in this particular macrophyte shape.

This negative interaction in a structurally complex habitat differs from the results of Swisher et al. (1998), who found bluegill sunfish (*L. macrochirus*) and libellulid dragonfly larvae (*E. simplicicollis*) had a positively non-additive impact at low densities of *Ceratophyllum demersum* analogues, which became additive at higher densities. The mayfly prey escaped dragonfly attacks by swimming away, which made them more vulnerable to bluegill predation at low densities where they could be easily detected, and hence there was a facilitative interaction between these predators at low macrophyte densities. Indeed, Swisher et al. (1998) predicted that the synergistic effects of multiple predators would be more apparent at lower macrophyte densities where it is easier to find and capture escaping prey. However, it must be remembered that the results of Swisher et al. (1998) were for macrophyte density, while ours were contingent on macrophyte shape.

Given that both macrophyte shape and macrophyte density contribute to habitat structure in vegetated systems, the results from both studies could be combined to predict a relationship between habitat structure and the impact of multiple predators (Fig. 1). This model hypothesises that as habitat structure becomes more complex, i.e. more structurally complex in shape and more dense in the amount of structure, the number of prey consumed by multiple predators decreases, leading to negative non-additivity. The results of Swisher et al. (1998) lie on the left of the relationship; the effects of multiple predators are positively non-additive (or facilitative) at low levels of habitat structure where prey are easier to detect and their avoidance behaviour can increase their risk of predation. Our results lie on the right of the relationship; the effects of multiple predators are non-additive where the habitat structure can interfere with a predator's ability to detect and capture prey, and can mediate predator–predator interactions. Fruitful avenues for research would include: (1) determining how such a hypothesised relationship would depend on one predator not being able to consume the other to any great degree, as this would be expected to influence the total number of prey consumed and thereby the combined impact of both predators; and (2) determining how such a hypothesised relationship would depend on the type and behaviour of prey.

This experiment illustrates the importance of testing both the quantitative (density) and qualitative (shape) components of habitat structure in order to understand the
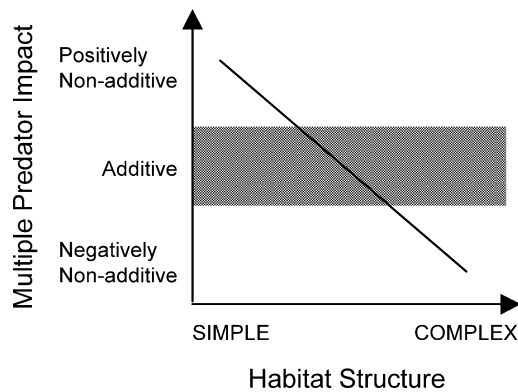
Fig. 1 Hypothesised relationship between habitat structural complexity, which incorporates both quantitative (density) and qualitative (shape) components of habitat structure, and the impact of multiple predators

mechanisms by which it may mediate trophic interactions. Not only can habitat structure influence the outcome of predator–prey interactions, but also the outcome of predator–predator interactions and thereby the combined impact of multiple predators. It is possible that the effects of predation in the field, and therefore the functional significance of predators in food webs, may depend on the type of habitat available, and that strong predatory effects may be more tightly coupled with structurally simple habitats (Power 1992). A field experiment exploring this hypothesis, with the expectation that pygmy perch would have the greatest direct and indirect effects in the structurally simple macrophyte, will be presented elsewhere.

References

Beck MW (2000) Separating the elements of habitat structure: independent effects of habitat complexity and structural components on rocky intertidal gastropods. J Exp Mar Biol Ecol 249:29–49

Beckerman AP, Uriarte M, Schmitz OJ (1997) Experimental evidence for a behaviour-mediated trophic cascade in a terrestrial food chain. Proc Natl Acad Sci U S A 94:10735–10738

Bettoli PW, Maceina MJ, Noble RL, Betsill RK (1992) Piscivory in largemouth bass as a function of aquatic vegetation abundance. N Am J Fish Manage 12:509–516

Carlisle DM, Hawkins CP (1998) Relationships between invertebrate assemblage structure, two trout species, and habitat structure in Utah mountain lakes. J N Am Benthol Soc 17:286–300

Chilton EWI (1990) Macroinvertebrate communities associated with three aquatic macrophytes (*Ceratophyllum demersum*, *Myriophyllum spicatum*, and *Vallisneria americana*) in Lake Onalaska, Wisconsin. J Freshwater Ecol 5:455–466

Coen LD, Heck KLJ, Abele LG (1981) Experiments on competition and predation among shrimps of seagrass meadows. Ecology 62:1484–1893

Coull BC, Wells JBJ (1983) Refuges from fish predation: experiments with phytal meiofauna from the New Zealand rocky intertidal. Ecology 64:1599–1609

Crowder LB, Cooper WE (1982) Habitat structural complexity and the interaction between bluegills and their prey. Ecology 63:1802–1813

Crowder LB, Squires DB, Rice JA (1997) Nonadditive effects of terrestrial and aquatic predators on juvenile estuarine fish. Ecology 78:1796–1804

Crowder LB, McCollum EW, Martin TH (1998) Changing perspectives on food web interactions in lake littoral zones. In: Jeppesen E, Sondergaard M, Sondergaard M, Christoffersen K (eds) The structuring roles of submerged macrophytes in lakes. Springer, Berlin Heidelberg New York, pp 240–249

Cyr H, Downing JA (1988) The abundance of phytophilous invertebrates on different species of submerged macrophytes. Freshwater Biol 20:365–374

Davies PE, Humphries P (1996) An environmental flow study of the Meander, Macquarie and South Esk Rivers, Tasmania. Department of Primary Industry and Fisheries

Dean RL, Connell JH (1987) Marine invertebrates in an algal succession. III. Mechanisms linking habitat complexity with diversity. J Exp Mar Biol Ecol 109:249–273

Dettmers JM, Wahl DH, Soluk DA, Gutreuter S (2001) Life in the fast lane: fish and foodweb structure in the main channel of large rivers. J N Am Benthol Soc 20:255–265

Diehl S (1988) Foraging efficiency of three freshwater fishes: effects of structural complexity and light. Oikos 53:207–214

Diehl S, Kornijow R (1998) Influence of submerged macrophytes on trophic interactions among fish and macroinvertebrates. In: Jeppesen E, Sondergaard M, Sondergaard M, Christoffersen K (eds) The structuring roles of submerged Macrophytes in lakes. Springer, Berlin Heidelberg New York, pp 24–46

Dionne M, Folt CL (1991) An experimental analysis of macrophyte growth forms as fish foraging habitat. Can J Fish Aquat Sci 48:123–131

Dionne M, Butler M, Folt C (1990) Plant-specific expression of antipredator behaviour by larval damselflies. Oecologia 83:371–377

Edgar GJ (1983) The ecology of south-east Tasmanian phytal animal communities. I. Spatial organization on a local scale. J Exp Mar Biol Ecol 70:129–157

Folsom TC, Collins NC (1984) The diet and foraging behaviour of the larval dragonfly *Anax junius* (Aeshnidae), with an assessment of the role of refuges and prey activity. Oikos 42:105–113

Gilinsky E (1984) The role of fish predation and spatial heterogeneity in determining benthic community structure. Ecology 65:455–468

Gotceitas V, Colgan P (1989) Predator foraging success and habitat complexity: quantitative test of the threshold hypothesis. Oecologia 80:158–166

Heads PA (1985) The effect of invertebrate and vertebrate predators on the foraging movements of *Ischnura elegans* larvae (Odonata: Zygoptera). Freshwater Biol 15:559–571

Heck KLJ, Crowder LB (1991) Habitat structure and predator–prey interactions in vegetated aquatic systems. In: Bell SS, McCoy ED, Mushinsky HR (eds) Habitat structure: the physical arrangement of objects in space. Chapman and Hall, London, pp 281–299

Heck KLJ, Orth RJ (1980) Seagrass habitats: the roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. In: Kennedy VS (ed) Estuarine perspectives. Academic Press, London, pp 449–464

Heck KLJ, Thoman TA (1981) Experiments on predator–prey interactions in vegetated aquatic habitats. J Exp Mar Biol Ecol 53:125–134

Heck KLJ, Wetstone GS (1977) Habitat complexity and invertebrate species richness and abundance in tropical seagrass meadows. J Biogeogr 4:135–142

Humphries P (1995) Life history, food and habitat of southern pygmy perch, *Nannoperca australis*, in the Macquarie River, Tasmania. Mar Freshwater Res 46:1159–11

Humphries P (1996) Aquatic macrophytes, macroinvertebrate associations and water levels in a lowland Tasmanian river. Hydrobiologia 321:219–233

Humphries P, Davies PE, Mulcahy ME (1996) Macroinvertebrate assemblages of littoral habitats in the Macquarie and Mersey Rivers, Tasmania: implications for the management of regulated rivers. Regul River 12:99–122

Jacobsen L, Perrow MR, Landkildehus F, Hjorne M, Lauridsen TL, Berg S (1997) Interactions between piscivores, zooplanktivores and zooplankton in submerged macrophytes: preliminary observations from enclosure and pond experiments. Hydrobiologia 342/343:197–205

James PL, Heck KLJ (1994) The effects of habitat complexity and light intensity on ambush predation within a simulated seagrass habitat. J Exp Mar Biol Ecol 176:187–200

Koperski P (1997) Changes in feeding behaviour of the larvae of the damselfly *Enallagma cyathigerum* in response to stimuli from predators. Ecol Entomol 22:167–175

Leber KM (1985) The influence of predatory decapods, refuge, and microhabitat selection on seagrass communities. Ecology 66:1951–1964

Lipcius RN, Eggleston DB, Miller DL, Luhrs TC (1998) The habitat–survival function for Caribbean spiny lobster: an inverted size effect and non-linearity in mixed algal and seagrass habitats. Mar Freshwater Res 49:807–816

Losey JE, Denno RF (1988) Positive predator–predator interactions: enhanced predation rates and synergistic suppression of aphid populations. Ecology 79:2143–2152

Main KL (1987) Predator avoidance in seagrass meadows: prey behaviour, microhabitat selection, and cryptic coloration. Ecology 68:170–180

Martin TH, Wright RA, Crowder LB (1989) Non-additive impact of blue crabs and spot on their prey assemblages. Ecology 70:1935–1942

McCoy ED, Bell SS (1991) Habitat structure: the evolution and diversification of a complex topic. In: McCoy ED, Bell SS, Mushinsky HR (eds) Habitat structure: the physical arrangement of objects in space. Chapman and Hall, London, pp 3–27

McIntosh AR, Peckarsky BL (1996) Differential behavioural responses of mayflies from streams with and without fish to trout odour. Freshwater Biol 35:141–148

McPeek MA, Peckarsky BL (1998) Life histories and the strengths of species interactions: combining mortality, growth, and fecundity effects. Ecology 79:867–879

Morin PJ (1995) Functional redundancy, non-additive interactions, and supply-side dynamics in experimental pond communities. Ecology 76:133–149

Nelson WG (1979) Experimental studies of selective predation on amphipods: consequences for amphipod distribution and abundance. J Exp Mar Biol Ecol 38:225–245

Nelson WG, Bonsdorff E (1990) Fish predation and habitat complexity: are complexity thresholds real? J Exp Mar Biol Ecol 141:183–194

Orr BK, Resh VH (1991) Interactions among aquatic vegetation, predators and mosquitoes: implications for management of *Anopheles* mosquitoes in a freshwater marsh. Proc Calif Mosq Vector Control Assoc 58:214–220

Orr BK, Resh VH (1992) Influence of *Myriophyllum aquaticum* cover on *Anopheles* mosquito abundance, oviposition and larval microhabitat. Oecologia 90:474–482

Persson L, Eklov P (1995) Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. Ecology 76:70–81

Pierce CL (1988) Predator avoidance, microhabitat shift, and risk-sensitive foraging in larval dragonflies. Oecologia 77:81–90

Polis GA (1989) Exploitation competition and the evolution of interference, cannabalism, and intraguild predation in age/size structured populations. In: Ebenman B, Persson L (eds) Size-structured populations. Springer, Berlin Heidelberg New York, pp 185–202

Power ME (1992) Habitat heterogeneity and the functional significance of fish in river food webs. Ecology 73:1675–1688

Rooke B (1986) Macroinvertebrates associated with macrophytes and plastic imitations in the Eramosa River, Ontario, Canada. Arch Hydrobiol 106:307–325

Ryer C (1988) Pipefish foraging: effects of fish size, prey size and altered habitat complexity. Mar Ecol Prog Ser 48:37–45

Savino JF, Stein RA (1982) Predator–prey interaction between largemouth bass and bluegills as influenced by simulated, submersed vegetation. Trans Am Fish Soc 111:255–266

Savino JF, Stein RA (1989) Behavioural interactions between fish predators and their prey: effects of plant density. Anim Behav 37:311–321

Sih A, Englund G, Wooster D (1998) Emergent impacts of multiple predators on prey. Trends Ecol Evol 13:350–355

Soluk DA (1993) Multiple predator effects: predicting combined functional response of stream fish and invertebrate predators. Ecology 74:219–225

Soluk DA, Collins NC (1988) Synergistic interactions between fish and stoneflies: facilitation and interference among stream predators. Oikos 52:94–100

Soluk DA, Richardson JS (1997) The role of stoneflies in enhancing growth of trout: a test of the importance of predator–predator facilitation within a stream community. Oikos 80:214–219

Stoner AW (1982) The influence of benthic invertebrates on the foraging behaviour of pinfish, *Lagodon rhomboides* (Linnaeus). J Exp Mar Biol Ecol 58:271–284

Stoner AW, Lewis FGI (1985) The influence of quantitative and qualitative aspects of habitat complexity in tropical seagrass meadows. J Exp Mar Biol Ecol 94:49–40

Swisher BJ, Soluk DA, Wahl DH (1998) Non-additive predation in littoral habitats: influences of habitat complexity. Oikos 81:30–37

Walsh EJ (1995) Habitat-specific predation susceptibilities of a littoral rotifer to two invertebrate predators. Hydrobiologia 313/314:205–211

Warfe DM (2003) The role of habitat structure in a freshwater food web. PhD dissertation. University of Tasmania

Werner EE, Gilliam JF, Hall DJ, Mittelbach GG (1983) An experimental test of the effects of predation risk on habitat use in fish. Ecology 64:1540–1548

Wilkinson L (1999) SYSTAT, version 9. SYSTAT, Evanston, Ill.