



Feeding dynamics of larval Pacific herring (*Clupea pallasii*) on natural prey assemblages: the importance of protists

LAURA E. FRIEDENBERG*, STEPHEN M. BOLLENS AND GRETCHEN ROLLWAGEN-BOLLENS

School of Earth and Environmental Sciences, Washington State University Vancouver, 14204 NE Salmon Creek Ave., Vancouver, WA 98686, USA

ABSTRACT

The role of protists in the diet of larval Pacific herring (*Clupea pallasii*) was examined in laboratory incubations conducted in May and June 2008 using a natural assemblage of microplankton (10–200 μm). Available prey consisted of protists (diatoms, dinoflagellates, aloricate ciliates, and loricate ciliates) and metazoans (trochophores, bivalve larvae, rotifers, copepod nauplii, and gastropod larvae). We used a prey enumeration technique that included soft-bodied heterotrophic protists (aloricate ciliates and athecate dinoflagellates) in the diet. We observed significant consumption of aloricate ciliates, loricate ciliates, bivalve larvae, dinoflagellates, and other available prey 73–200 μm . Clearance rates ($\text{mL larva}^{-1} \text{h}^{-1}$) and an electivity index E^* were used as measures of prey selectivity. The herring larvae showed strong selection for bivalve larvae and 73–200 μm available prey; however, across all valid experiments, protist prey were selected for at rates comparable to metazoans. Ingestion rates ($\mu\text{g C larva}^{-1} \text{h}^{-1}$) showed that the majority of larval carbon intake was from diatoms and aloricate ciliates. The results of this study illustrate that there is a direct trophic link between larval herring and the microbial food web, and protists may make up a substantial portion of the larval fish diet, possibly alleviating food limitation.

Key words: clearance rate, *Clupea pallasii*, ingestion rates, larval fish feeding, Pacific herring, protist, selectivity

INTRODUCTION

Once hatched, fish are at their most vulnerable and experience the highest degree of mortality during the larval stage after the yolk sac is absorbed (Houde, 1989). A major source of larval death is thought to be starvation due to feeding failure or inadequate nutritional quality of prey items (Hjort, 1914; Cushing, 1975; Lasker, 1975), although much of the evidence for this comes from laboratory-based studies (reviewed in Leggett and Deblois, 1994). First-feeding larval fish are traditionally recognized as zooplanktivores, consuming primarily metazoans (multicellular eukaryotes) such as calanoid copepod nauplii, copepodites, and invertebrate larvae (Arthur, 1976; Last, 1978a,b; Munk and Kiorboe, 1985). However, the diet of larval fish is determined by prey availability, prey escape response, and mouth gape size in relation to prey size (Checkley, 1982; Figueiredo *et al.*, 2007). If larvae do not have access to, or cannot ingest, large, nutritious prey items, they may consume smaller, slower, nutritionally poor, but more abundant prey such as protists (single-celled autotrophic, heterotrophic and mixotrophic eukaryotes, e.g., diatoms, ciliates, and dinoflagellates) to survive. Consumption of protists may alleviate food limitation of larval fish (Stoecker and Capuzzo, 1990; Hunt von Herbing and Gallager, 2000), and may allow the larvae to grow and survive until they either encounter larger zooplankton or their gape size can accommodate large prey (Nagano *et al.*, 2000; Figueiredo *et al.*, 2007). Additionally, larval fish may have a poor digestive capacity at the onset of feeding, and protists may provide a food source that is assimilated more easily than copepodites and invertebrate nauplii (Reitan *et al.*, 1998).

An increasing body of literature identifies larval fish as a link between metazoan and microbial food webs. Laboratory and field studies involving gut content analysis of first-feeding larval fish show that a wide variety of prey items are consumed, including protists, but as fish larvae grow, they become more selective and target larger prey items (Hardy, 1924; Arthur, 1976; Checkley, 1982; Bollens and Sanders, 2004). For instance, tintinnid ciliates (heterotrophic protists with a hard lorica surrounding the body) have been found in the guts of larval fish in the English Channel and

*Correspondence. e-mail: laura.friedenberg@email.wsu.edu

Received 17 December 2010

Revision version accepted 8 October 2011

the North Sea (Last, 1978a,b), the San Francisco Estuary (Bollens and Sanders, 2004), the Irish Sea (Figueiredo *et al.*, 2007), and Tosa Bay, Japan (Fukami *et al.*, 1999). Laboratory investigations using gut content analysis methods have also shown that larval fish may consume protists (Figueiredo *et al.*, 2007). However, examination of gut contents only reveals empty lorica of tintinnid ciliates or, at best, unidentifiable soft-bodied protist cells, as evidence of protist consumption. These laboratory and field studies draw attention to an aspect of larval fish diet and food limitation that has been overlooked previously (i.e., the role of protists) but they do not provide a quantitative measure of the degree to which larval fish may select for protist taxa or their rates of ingestion of these prey.

Gut content analysis is only adequate for identifying prey items with hard parts, such as lorica (ciliates) or theca (dinoflagellates). Organisms that lack hard parts (e.g., aloricate ciliates and athecate dinoflagellates) are rapidly digested and are not identifiable in the gut contents (Spittler *et al.*, 1990; Nagano *et al.*, 2000; Figueiredo *et al.*, 2007). Even if all prey items were discernible in the gut contents, there may be a high percentage of empty guts sampled as a result of regurgitation and/or defecation during the sampling process, which could lead to an underestimation of prey available to fish larvae (Figueiredo *et al.*, 2005; Pepin and Dower, 2007).

Several targeted studies have found heterotrophic protists in the guts of larval fish by labeling protists with protist-specific immunofluorescent antibody probes (Ohman *et al.*, 1991), the fluorescent DNA-specific stain DAPI (Lessard *et al.*, 1996), and fluorescent microspheres (Nagano *et al.*, 2000). Protist consumption by larval fish has also been observed by tracing lipid biomarkers (Rossi *et al.*, 2006), video observations of feeding events (Hunt von Herbing and Gallagher, 2000), and detection of aloricate ciliate DNA in guts of larval fish collected from the field using epifluorescence microscopy (Fukami *et al.*, 1999). However, these studies were limited in their results and did not address selectivity or ingestion of the larvae on protist prey, and the prey assemblage in the laboratory studies consisted only of protists.

Another approach, used in crustacean zooplankton studies but not thus far with larval fish, is to examine natural assemblages of both soft-bodied and hard-bodied prey before and after incubation with predators (e.g., Rollwagen-Bollens and Penry, 2003; Gifford *et al.*, 2007). This indirect methodology, which we have adapted for this study, allows for the inclusion of soft-bodied heterotrophic protists in the diet and the

calculation of selectivity and ingestion rates for multiple prey taxa that the larvae would encounter in the field. To date there have been no studies investigating larval Pacific herring (*Clupea pallasii*) diet using methods that incorporate soft-bodied heterotrophic protists.

In this study our objectives were to experimentally determine (i) what first feeding Pacific herring larvae consume when presented with a natural assemblage of prey, as well as (ii) the selectivity and (iii) the ingestion rates of the larvae on their prey. Pacific herring were chosen because they are of great ecological and economical value, but many populations are no longer a viable fishery resource due to over-exploitation, predation pressure, and habitat degradation (Hershberger *et al.*, 2005; Vdovin and Chernouvanova, 2006; Chimura *et al.*, 2009). More generally this study attempts to clarify the planktonic food web dynamics between lower trophic levels (such as protists) and larval Pacific herring.

MATERIALS AND METHODS

Larval herring feeding experiments

We conducted six feeding experiments in May and June of 2008 that examined the role of microplankton (10–200 μm) in the diet of larval Pacific herring from Puget Sound, WA, using modified methods from Rollwagen-Bollens and Penry (2003) and Gifford *et al.* (2007). We chose to define microplankton as 10–200 μm instead of the standard 20–200 μm range as this better reflects the size distribution of potential prey items in the experimental area. These experiments were conducted at the United States Geological Survey (USGS) Marrowstone Marine Field Station located in Nordland, WA. Three of the experiments were conducted in May 2008, and another three experiments were conducted in June 2008. Within each month, a cohort of larvae grown in the laboratory was used, and experiments were conducted within 24–48 h of each other to minimize the potential effects of differential larval size and growth on feeding. In addition, three preliminary experiments were conducted in June 2007 that tested methodology, including appropriate prey density, predator density, and feeding conditions for the larvae. Larval herring used in all experiments were collected as embryos from Holmes Harbor, WA (May) and Cherry Point, WA (June) by the Washington Department of Fish and Wildlife. After hatching, larvae were reared in flow-through 760-L tanks that were supplied with filtered ambient seawater from Puget Sound (salinity 29,

10–11°C), and were fed marine rotifers (*Brachionus plicatilis*), brine shrimp (*Artemia franciscana*), and concentrated algae (*Isochrysis* sp., *Nannochloropsis* sp.).

Seawater containing a natural assemblage of prey was collected from the surface of Puget Sound off a nearby dock on Marrowstone Island. Twenty 1-L jars were covered with 200- μ m mesh and dipped into a bucket of freshly collected seawater. The 1-L jars were covered with duct tape on the sides to create contrast and enhance larval feeding, and the bottoms of the jars were left uncovered to allow for light penetration. In the laboratory, the contents of the jars were placed into a large bucket to homogenize the plankton. A 1-L subsample of seawater was filtered over a 73- μ m sieve and the sieve contents were enumerated under a dissecting microscope to ensure that the density of the plankton was appropriate for detecting a decrease in prey (based on preliminary experiments). This prey density range was approximately 100 metazoans per liter in the 73–200- μ m size range. The 1-L jars were refilled with 500 mL of the homogenized natural seawater and 500 mL of filtered seawater that did not contain prey items, creating a 1 : 1 dilution of natural and filtered seawater, so as to attain the desired prey density in order to detect a feeding response. Two experimental jars without larval fish predators served as 'initial controls' and were preserved immediately (described below). Four additional jars without predators served as 'final control' chambers, and 12 jars served as 'treatment' chambers. The treatments consisted of triplicate jars that contained 2, 4, 8, or 16 herring larvae. These four densities of herring larvae were used to ensure the detection of prey consumption, and not to detect predator density effects.

Larval herring (19–22 days post hatch, 11–13 mm TL) were starved for 15 h prior to each experiment. Approximately 200 larvae were collected from the housing tanks and randomly sorted into 24 30-mL cups containing 10 mL of seawater and no more than four larvae per cup to reduce crowding and stress. From the 30-mL cups, the appropriate number of larvae (2–16, based on treatment) were placed into the 1-L chambers containing diluted seawater (10.3°C, 29 psu) under a combination of natural and fluorescent light ($1.8 \mu\text{E m}^{-2} \text{s}^{-1}$). The mouth of each jar was covered with Parafilm to eliminate bubbles, capped, and placed on a rotating (1 rpm) plankton wheel to keep the plankton in suspension for the duration of each experiment. The jars were left to rotate on the plankton wheel for 6.5 h. The incubation time was chosen based on preliminary experiments, direct feeding studies (Checkley, 1982; Munk and Kiorboe, 1985), search volume (Munk and Kiorboe, 1985), and

bioenergetics (Bollens, 1988), to ensure that a detectable level of feeding would occur. At the end of the incubation period, the jars were removed from the plankton wheel and the seawater and larval herring were preserved. Larval herring were placed via pipette into small vials, anesthetized with MS-222, and preserved with 10% formalin. No larval herring mortality occurred during any of the experiments. cursory observations of larval herring guts were made after each experiment to confirm that prey had been consumed, but for reasons noted in the Introduction, such observations did not allow confirmation of consumption of most protist prey types.

Two methods were used for the preservation of seawater and plankton in the treatment and control chambers. To detect the presence of protist plankton (10–200 μ m), 200 mL of seawater from each chamber was preserved in 5% acid Lugol's solution. Larger metazooplankton (73–200 μ m) were separated from the remaining 800 mL by filtration through a 73- μ m sieve and preserved in 10% formalin and filtered seawater solution.

Cell counts and biomass estimations

To enumerate protists in the Lugol-preserved samples (10–200 μ m), 10–15 mL aliquots from each sample bottle were settled overnight in Utermöhl chambers. The entire contents of the chamber were enumerated using an Olympus CK40 inverted microscope at 200 $\times$ magnification. Using an ocular micrometer, the size (length and width) and morphology (shape) of each prey item was recorded. Prey items were grouped into one of the following major prey categories: diatoms, dinoflagellates, aloricate ciliates, and loricate ciliates. Individuals were further placed into one of the following size categories; 10–30, 30–73, and 73–200 μ m. Individual cells of chain-forming diatom genera, such as *Skeletonema*, *Thalassiosira*, and *Chaetoceros*, were also enumerated and placed into the 10–30 μ m size category.

To enumerate metazoans (73–200 μ m) in the formalin-preserved samples, the entire contents of the sample jar were counted on a Leica MZ6 dissecting microscope at 10 $\times$ magnification and prey items were grouped into the following categories: trochophores, bivalve larvae, rotifers, copepod nauplii, and gastropod larvae. The size and morphology of each prey item were recorded. Protist prey in these samples were not counted. Protist and metazoan prey density (cells mL^{-1}) for each sample were combined. The biovolume for all prey types was calculated based on geometric shape (Hillebrand *et al.*, 1999) and carbon biomass was estimated using a biovolume–biomass conversion for

the protist plankton (Menden-Deuer and Lessard, 2000) and the metazoan plankton (Yamaguchi *et al.*, 2005).

Selectivity and feeding rate calculations

Clearance rates ($\text{mL larva}^{-1} \text{h}^{-1}$) and ingestion rates ($\mu\text{g C larva}^{-1} \text{h}^{-1}$) were calculated using the equations of Marin *et al.* (1986). Clearance rates were calculated based on changes in prey abundance over the incubation relative to growth of prey in control bottles. Similarly, ingestion rates were calculated based on the change in carbon biomass over the incubation compared to growth in controls. Clearance rates are used here as the primary measure of selective feeding by the larval herring consumers, and are the preferred method for estimating diet selection in enclosed incubation experiments such as those used in this study (Frost, 1972; Marin *et al.*, 1986; Rollwagen-Bollens and Penry, 2003). However, for comparison we also calculated an electivity index (E^* , Vanderploeg and Scavia, 1979a,b) as a second measure of larval herring preference, following the approach described in Rollwagen-Bollens and Penry (2003). As reviewed by Lechowicz (1982) and Confer and Moore (1987), E^* is sufficiently stable to accommodate both changes in relative abundance of food types and the presence of rare prey types.

Data analysis

Statistical analyses were conducted using SIGMASTAT 3.5 (Systat, San Jose, California, USA). One-way ANOVA with equal variance tests (Levene's test) were conducted for each experiment and treatment to determine if there was a significant difference ($P < 0.05$, Tukey 5 multiple range test) in the number of each prey taxon, as well as the number of total prey, between the final control and treatment bottles. Our criteria for accepting any experiment as valid were: (i) a significant reduction ($P < 0.05$) in at least one prey

taxon in any size category between final controls and treatments, and (ii) the abundance of a significantly reduced group in the final controls contained a minimum of five individuals per liter. One-way ANOVAs were conducted for each experiment separately because initial prey abundance was significantly different among experiments, and thus experiments could not be compared to each other.

A two-way ANOVA with equal variances (Levene's test) was conducted on the grouped abundance data to determine whether experiment, predator density, or an interaction between the two, affected final prey abundance. Finally, one-way ANOVA tests were conducted on the clearance and ingestion rates for each prey type calculated from each treatment. A significant difference ($P < 0.05$) in clearance rates between any two or more prey categories within a treatment was interpreted as selective feeding by the fish larvae. On rare occasions, our calculations of clearance rate resulted in negative values. In those instances, we excluded negative clearance rates from our subsequent analyses to allow us to focus on the direct effects of larval herring on their prey. Negative clearance rates on a particular prey result when that prey increases in abundance (relative to the controls) during the incubation, such as might occur when its competitor is consumed by the larval fish. However, such indirect effects of larval fish predation, while very interesting and potentially important, were beyond the scope of this study.

RESULTS

Significant reductions ($P < 0.05$) in major prey taxa, size classes within taxa, or size categories regardless of taxa were detected in six treatments within three of our experiments and were thus considered valid (Table 1). The other three experiments did not yield statistically significant differences between treatments and controls, for unknown reasons, such as larval stress

Table 1. Significant reductions in prey categories in treatments versus controls during larval herring feeding experiments. Experiments 1 and 2 took place in May 2008 and experiment 3 in June 2008.

	Experiment		
	1	2	3
Treatment			
4-Herring	NS	Aloricate ciliates*	Bivalve larvae ***
8-Herring	NS	Loricata ciliates*	Bivalve larvae ***
16-Herring	73–200 μm dinoflagellates*	Aloricate ciliates*	NS
	73–200 μm total prey**	30–73 μm aloricate ciliates*	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. NS, no significant results.

or other factors that we did not measure. From this point on, our results refer only to treatments within the three valid experiments. In experiment 1, there was a significant decrease in total available prey regardless of taxa in the 73–200 μm size category, and a significant decrease in 73–200 μm dinoflagellates (Table 1). In experiment 2, there was a significant decrease in abundance of aloricate ciliates in two treatments, and of loricate ciliates in one treatment (Table 1). There was also a significant decrease in 30–73 μm aloricate ciliates (Table 1). In experiment 3, there was a significant decrease in abundance of bivalve larvae in two treatments (Table 1).

The results of the two-way ANOVA comparing total final prey abundance versus experiment and treatment (fish density) showed that final prey abundance was significantly different among valid experiments ($P < 0.001$), but that treatment (fish density) did not significantly affect final prey abundance ($P = 0.991$). The two-way ANOVA revealed that there was no significant interaction between experiment and treatment among valid experiments. We

concluded that final prey density was independent of the number of predators in each treatment, and therefore predator density effects are not discussed further. Initial controls and final controls were also compared, and there were no significant differences between prey abundance in these controls.

Available prey composition, size, abundance, and biomass

Available protist prey consisted of diatoms, dinoflagellates, aloricate ciliates, and loricate ciliates. Available metazoan prey consisted of trochophore larvae, bivalve larvae, rotifers, copepod nauplii, and gastropod larvae. The protist prey assemblage was three orders of magnitude higher in abundance than the metazoan assemblage (Fig. 1a). Total carbon biomass ($\mu\text{g C mL}^{-1}$) of the prey was not significantly different among experiments ($P = 0.100$), and the majority of the biomass was from diatoms, dinoflagellates, aloricate ciliates, and copepod nauplii (Fig. 1b). Overall, the protist prey accounted for 99.9% of the mean abundance (ind. mL^{-1}) and 75.6% of the mean biomass ($\mu\text{g C mL}^{-1}$) for all experiments.

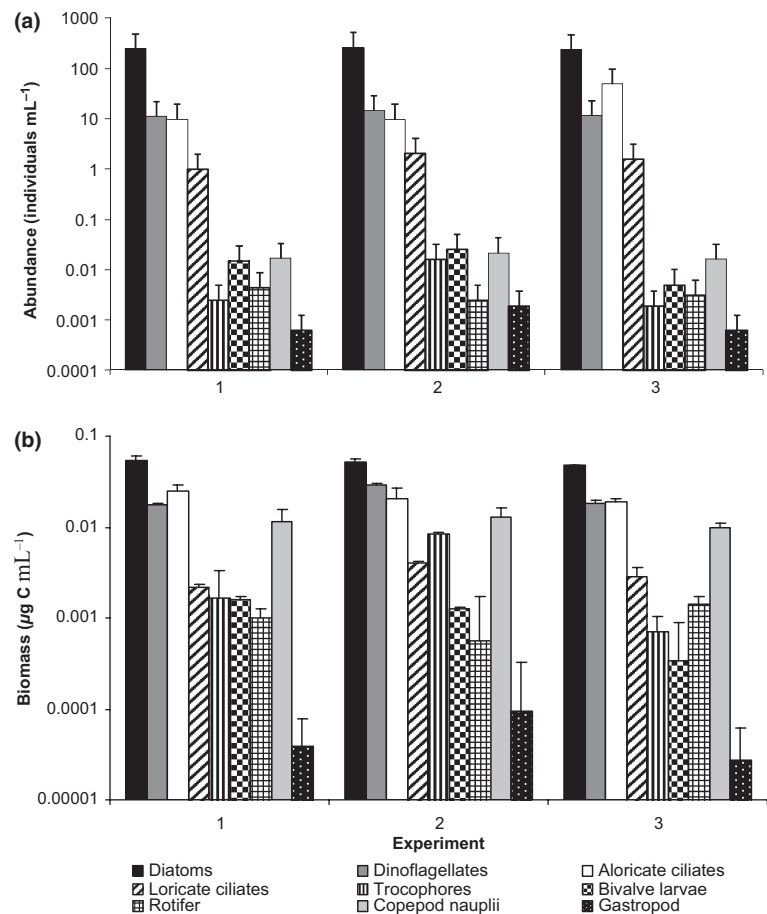


Figure 1. Mean initial prey density (individuals mL^{-1} , a) and carbon biomass ($\mu\text{g C mL}^{-1}$, b) of prey available to the larval herring during feeding experiments. Experiments 1 and 2 took place in May 2008 and experiment 3 in June 2008.

Clearance rates and electivity indices

Selection based on prey taxonomic composition. Clearance rates ($\text{mL larva}^{-1} \text{h}^{-1}$) and electivity indices (E^*) were used as measures of prey selection in treatments within valid experiments. In experiment 1, copepod nauplii were cleared at a rate of $8.2 \pm 1.9 \text{ mL larva}^{-1} \text{h}^{-1}$, whereas all other prey taxa were cleared at rates lower than $3 \text{ mL larva}^{-1} \text{h}^{-1}$ (Fig. 2a), although no significant differences among clearance rates were calculated ($P = 0.160$). In experiment 2, aloricate and loricate ciliates were cleared at rates ranging from 2.1 to $15.2 \text{ mL larva}^{-1} \text{h}^{-1}$ (Fig. 2b); however, there were no significant differences among clearance rates ($P = 0.124$), suggesting that heterotrophic protists were selected for at rates comparable to those of metazoans in each of the three fish treatments. In experiment 3, the highest clearance rates were calculated for bivalve larvae in two treatments (Fig. 2c), and the clearance rate for bivalve larvae in the 2-herring treatment was significantly higher ($P = 0.025$) than the clearance rates for other prey,

suggesting strong selection for this prey taxon. No significant difference in selectivity for any prey taxon was evident in the eight-herring treatment (Fig. 2c).

The electivity indices calculated from the same experiments and treatments as described above were generally aligned with the clearance rate estimations of larval herring diet preferences (Fig. 3). Larval herring electivity was positive most often for invertebrate larvae, and was either negative or not significantly different from zero for most protists (Fig. 3).

Selection based on prey size. Clearance rates were also used to assess prey selection among three size categories regardless of prey taxa: 10–30, 30–73, and 73–200 μm . In the two experiments in which prey within a size category were significantly reduced (Table 1), the ANOVA results showed that in experiment 1, the 73–200 μm prey were cleared at a rate significantly higher ($P = 0.023$) than in the 10–30 and 30–73 μm categories (Fig. 4a), suggesting strong selection for 73–200 μm prey by the larval herring. In experiment 2, no significant differences in clearance

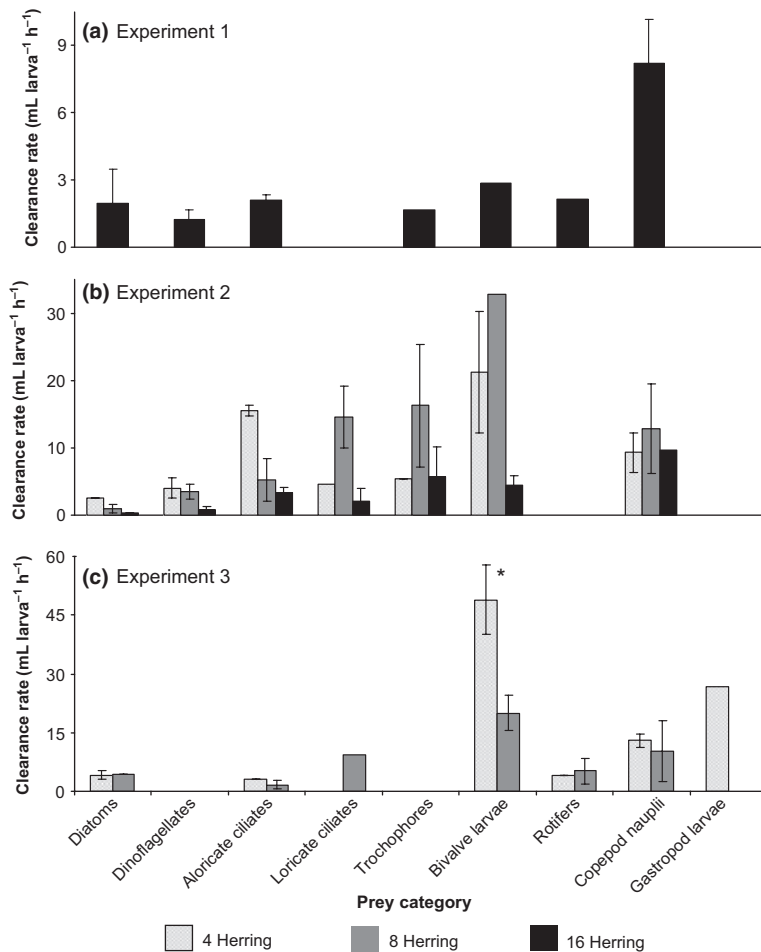


Figure 2. Clearance rates ($\text{mL larva}^{-1} \text{h}^{-1}$) for prey taxa in experiment 1 (a), experiment 2 (b) and experiment 3 (c) for treatments containing 4, 8 and 16 herring. Error bars: $\pm 1 \text{ SE}$. * $P < 0.05$.

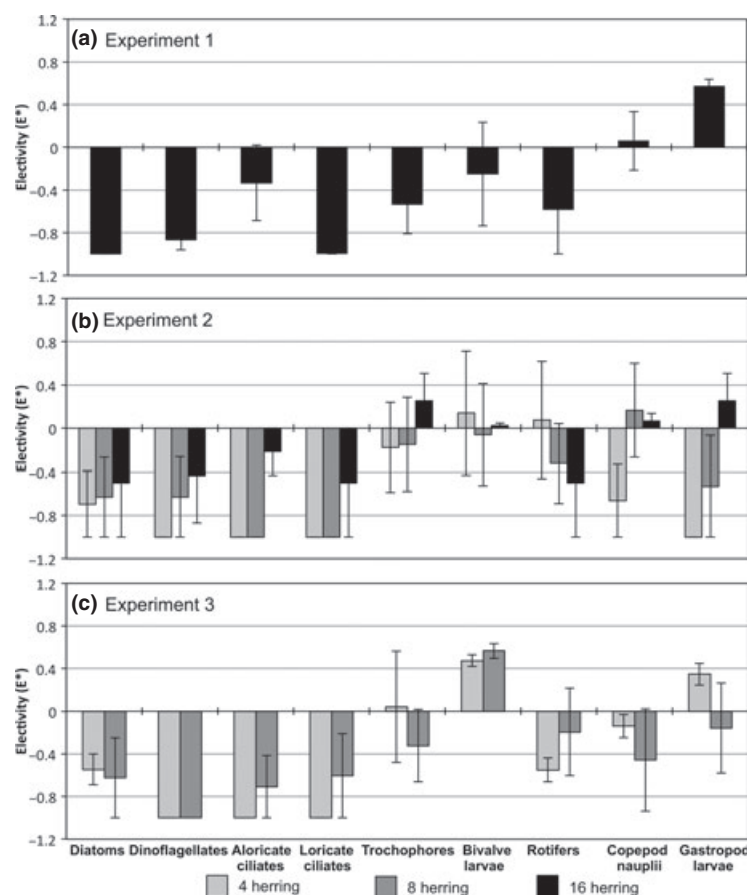


Figure 3. Electivity (E^*) indices for prey taxa in experiment 1 (a), experiment 2 (b), and experiment 3 (c) for treatments containing 4, 8, and 16 herring. Error bars: ± 1 SE.

rates were calculated for prey within any size category ($P = 0.095$; Fig. 4b). However, given the statistical power in experiment 2 ($P = 0.345$), greater replication may well have resulted in a truly significant ($P < 0.05$) result.

We also examined larval herring selection on individual prey taxa within each size category. Positive clearance rates were observed on a variety of taxa within each size category, ranging from $0.7 \text{ mL larva}^{-1} \text{ h}^{-1}$ for diatoms to $10.4 \text{ mL larva}^{-1} \text{ h}^{-1}$ for dinoflagellates. However, in neither experiment 1 nor experiment 2 were there any statistically significant differences among clearance rates for individual prey taxa based on size ($P = 0.245$, Fig. 5a; $P = 0.192$, Fig. 5b). Patterns of electivity indices were again in good agreement with clearance rates, based on both overall size categories and prey taxa within each size range (data not shown).

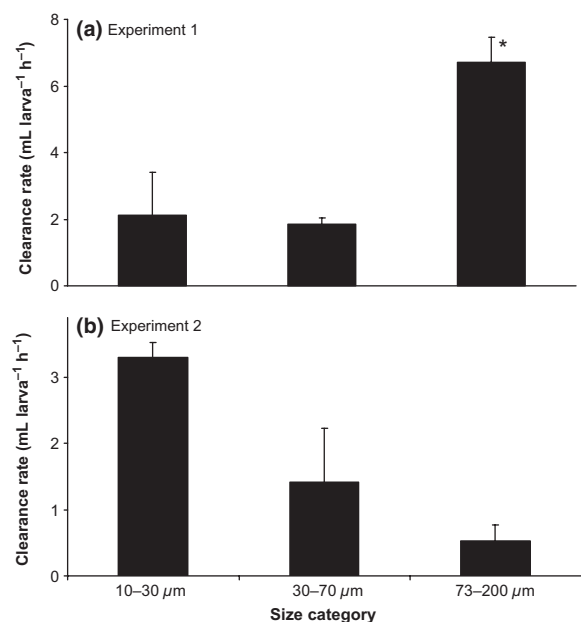
Ingestion rates

Major prey taxa. Ingestion rates of carbon biomass ($\mu\text{g C larva}^{-1} \text{ h}^{-1}$) were quantified in valid

experiments. In experiment 1, the ingestion rate for aloricate ciliates was significantly higher than for other prey taxa ($P = 0.028$), suggesting that aloricate ciliates contributed a substantial amount of carbon to the larval herring diet (Fig. 6a). In experiment 2, aloricate ciliate carbon was ingested at rates comparable to copepod nauplii carbon in the 8- and 16-herring treatments; however, in the 4-herring treatment, diatom carbon was ingested at a rate significantly higher ($P = 0.039$) than other prey taxa (Fig. 6b). In all treatments of experiment 2, dinoflagellates, loricata ciliates, trochophores, and bivalve larvae carbon was ingested at rates close to zero (Fig. 6b). In experiment 3, the ingestion rate for aloricate ciliates in the 8-herring treatment was significantly higher than for all other taxa ($P = 0.033$; Fig. 6c).

Prey size. Ingestion of carbon biomass based on prey size regardless of taxa was quantified for the three size categories: 10–30, 30–73, and 73–200 μm . In experiment 1, ingestion rates for all three size categories were similar, and no significant differences

Figure 4. Clearance rates ($\text{mL larva}^{-1} \text{h}^{-1}$) in experiment 1 (a) and experiment 2 (b) for prey size categories in treatments containing 16 herring. Error bars: ± 1 SE. * $P < 0.05$.



among prey sizes were observed ($P = 0.124$; Fig. 7a). In experiment 2, the ingestion rate for 73–200 μm prey was significantly higher than rates for the other two size categories ($P = 0.038$, Fig. 7b).

Ingestion rates for different prey taxa within the three size categories showed that in experiment 1, protist and metazoan carbon were consumed at comparable (statistically indistinguishable) rates (Fig. 8a). In this experiment, diatom and dinoflagellate carbon were ingested at intermediate rates, whereas carbon from metazoans and loricate ciliates were ingested at low rates. In experiment 2, the fish larvae consumed a broad range of prey types, as no significant differences in ingestion rates were observed for any particular prey taxon in any size category (Fig. 8b).

DISCUSSION

Larval herring diet and selectivity

Although copepod nauplii and copepodites have traditionally been recognized as important in the diet of larval fish, the results of this study suggest that heterotrophic protists may contribute substantially to their diet as well. Previous diet studies have shown that larval fish may consume loricate ciliates (Last, 1978a,b; Fukami *et al.*, 1999; Bollens and Sanders, 2004; Figueiredo *et al.*, 2005) and bivalve larvae (Checkley, 1982); however, to our knowledge there

have been no studies that have found larval Pacific herring to feed on soft-bodied heterotrophic protists when larvae have been presented with an unaltered, natural assemblage of prey available in the field.

Studies that have examined soft-bodied heterotrophic protist prey items in the diet of larval fish have relied on modified gut analysis or observational methods in the laboratory. For instance, Nagano *et al.* (2000) found a single taxon of cultured ciliates (*Euplotes* sp.) labeled with fluorescent microspheres in the guts of surgeonfish larvae (*Paracanthurus hepatus*), whereas unlabeled ciliates were never identified in the gut contents. Similarly, Lessard *et al.* (1996) detected several species of heterotrophic protists and autotrophic protists that were live-stained with 4',6-diamidino-2-phenylindole stain (DAPI) in the guts of larval Pollock (*Theragra chalcogramma*). Ohman *et al.* (1991), using detection of protist-specific immunofluorescent antibody probes, described predation of aloricate ciliates (*Strombidium* sp.) by first-feeding northern anchovy larvae. Hunt von Herbing and Gallager (2000) used video observations to confirm that larval Atlantic cod (*Gadus morhua*) prey upon a mono-culture of aloricate ciliates (*Balanion* sp.) Additionally, Nakagawa *et al.* (2007) used a food removal method to estimate the carbon flow between Pacific bluefin tuna larvae (*Thunnus orientalis*) and heterotrophic nanoplankton.

Larval fish feeding on protists has also been observed for larvae collected in the field. Fukami *et al.* (1999) used epifluorescence microscopy to detect DAPI-stained DNA of flagellate-like and ciliate-like cells in the guts of larval fish from Tosa Bay, Japan. In the Irish Sea, heterotrophic protists were estimated to constitute up to 35% of the diet of Atlantic herring larvae, and 6–9% for other fish larvae (Figueiredo *et al.*, 2005). In Conception Bay, Newfoundland, stable ^{15}N isotopes indicated that larval witch flounder (*Glyptocephalus cynoglossus*) and capelin (*Mallotus villosus*) fed significantly on autotrophic and heterotrophic protists, which contrasted with the results of stomach contents (Pepin and Dower, 2007). These studies showed a greater prevalence of protists in larval fish diet than previous diet studies based on traditional methods that under-sample protists.

These studies were able to show that different species of larval fish from diverse geographical areas could consume soft-bodied heterotrophic protists, although these studies did not calculate prey preference using a selectivity index. If larval fish only have access to or are only able to consume one type of prey, they may have to exploit that resource to survive. However, if larvae are presented with a variety of prey that includes

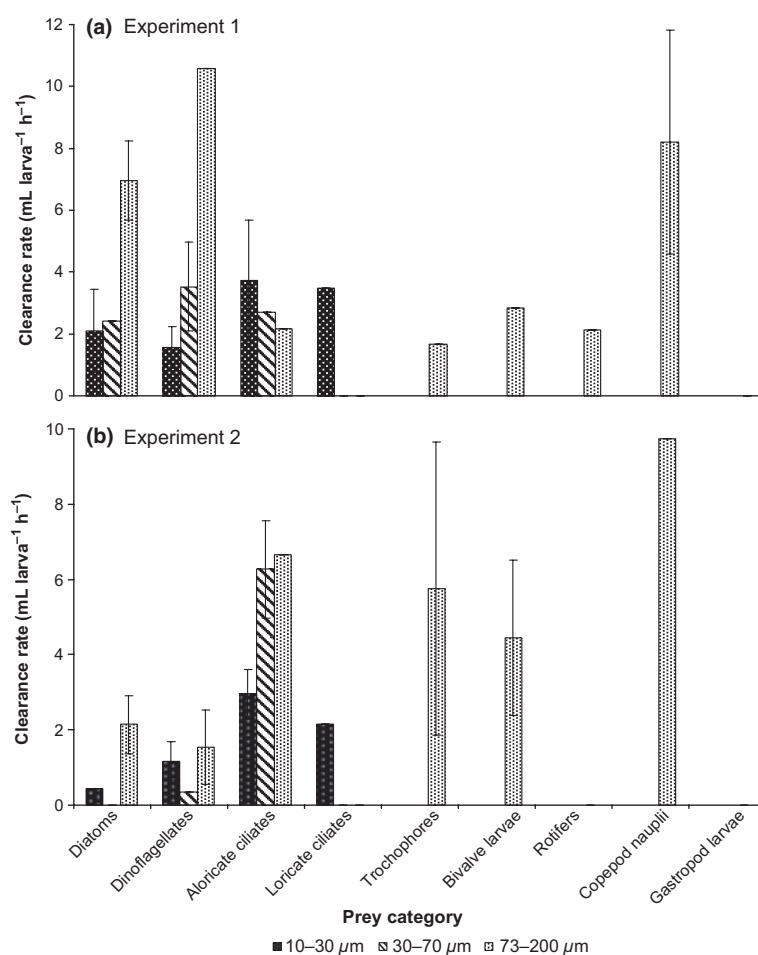


Figure 5. Clearance rates ($\text{mL larva}^{-1} \text{h}^{-1}$) in experiment 1 (a) and experiment 2 (b) for taxa within size categories in treatments containing 16 herring. Error bars: ± 1 SE.

phytoplankton, autotrophic and heterotrophic protists, copepod nauplii, and invertebrate larvae, then they may have the ability to discriminate between prey items. The methodology that we used in this study allowed us to determine not only diet composition of the larvae, but prey preference and ingestion rates as well.

Clearance rate ($\text{mL larva}^{-1} \text{h}^{-1}$) was used in this study as the primary measure of selectivity and is conceptually known as the intensity of larval search for prey based on the volume of water that a larva 'cleared' of prey within a period of time (Frost, 1972; Marin *et al.*, 1986). The calculations are based on the ratio of prey abundance remaining in the final treatments to initial abundance, and include cell growth in the absence of grazers in the controls (Rollwagen-Bollens and Penry, 2003). Clearance rate therefore illustrates the direct impact of larval herring on their prey in relation to prey availability.

In two of our three valid experiments, clearance rates were not significantly different for protists and metazoans (Fig. 2a,b), indicating that larval herring did not select for 'traditional' prey items, such as

bivalve larvae and copepod nauplii, over protists. Herring are visual predators and may select food based on size, contrast, and movement (Checkley, 1982; Figueiredo *et al.*, 2007). Thus bivalve larvae, although occurring in low concentrations, may have been consumed selectively in experiment 3 because of their larger size, slower movement, greater contrast, and minimal escape response. Additionally, our exclusion of negative clearance rates (see Materials and Methods), which was only necessary in a very small number of cases, could have led to an overestimation of clearance rate on that prey type in those rare instances. However, we believe that this is the best estimate of the direct effects of larval fish on their prey, as opposed to indirect effects that have ramifications for other components of the food web (and which were beyond the scope of this study).

Larval herring ingestion

The ingestion rates ($\mu\text{g C larva}^{-1} \text{h}^{-1}$) calculated in this study suggest that although metazoans contribute a substantial amount of carbon to the diet of larval

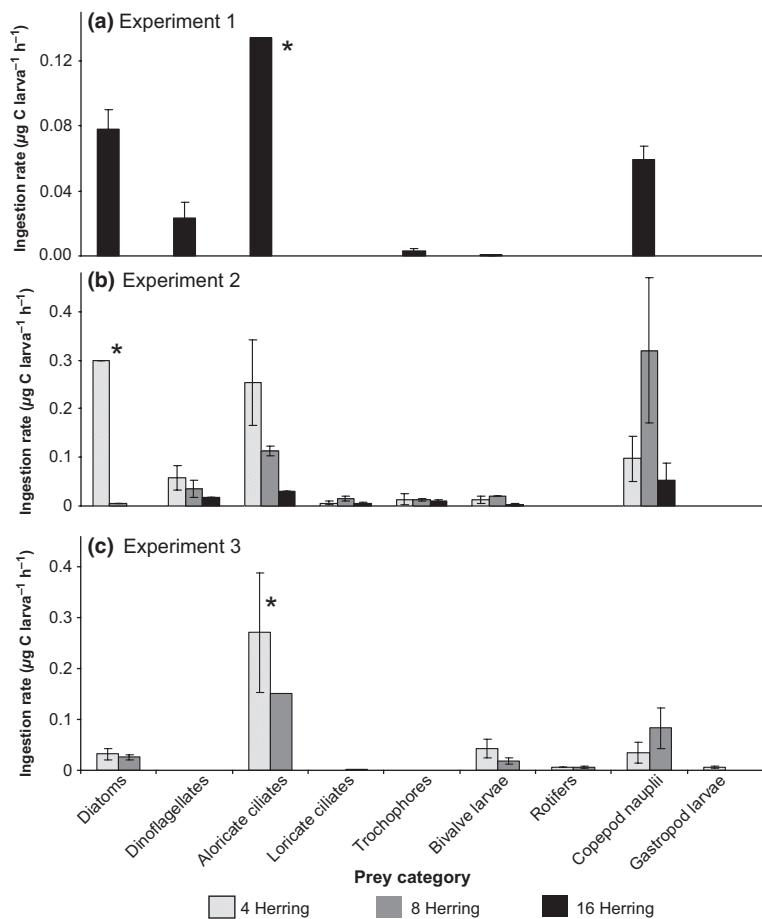
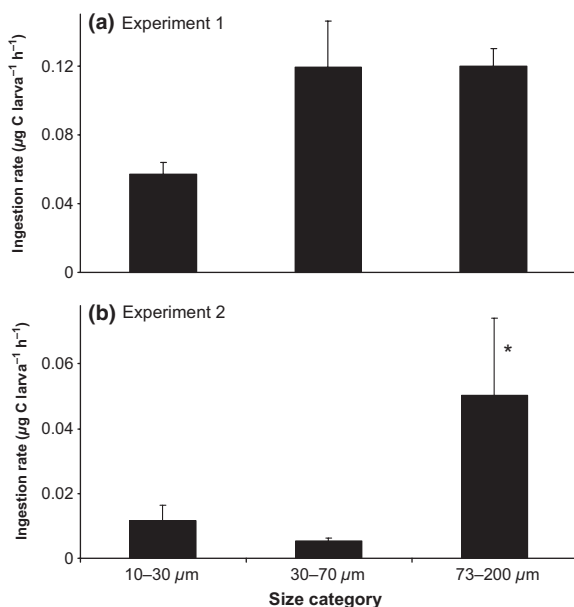


Figure 6. Ingestion rates ($\mu\text{g C larva}^{-1} \text{h}^{-1}$) for prey taxa in experiment 1 (a) experiment 2 (b) and experiment 3 (c) in treatments containing 4, 8 and 16 herring. Error bars: ± 1 SE. * $P < 0.05$.

Figure 7. Ingestion rates ($\mu\text{g C larva}^{-1} \text{h}^{-1}$) for prey size in experiment 1 (a) and experiment 2 (b) in treatments containing 16 herring. Error bars: ± 1 SE. * $P < 0.05$.



herring, protists may provide comparable amounts, and in some cases the majority, of carbon ingested (Fig. 6). Previous studies have found that larval fish are able to detect and ingest ciliates at lower densities than those found in marine environments (<20 ciliates mL^{-1}). For example, in the Irish Sea, naked and tintinnid ciliates were rare in the field but were detected in the diet of larval fish (Figueiredo *et al.*, 2007). Furthermore, larval fish must consume about 20% of their body carbon per day to sustain growth, and the upper density range of ciliates in the field may be high enough to meet this requirement (Lynn and Montagnes, 1991; Figueiredo *et al.*, 2007).

It is important to recognize the limitations of small-scale incubations compared with field conditions. Larvae reared in the laboratory require higher densities of food to grow, and ingestion rates in the laboratory are most likely affected primarily by time spent on handling of the prey, including pursuit, capture, consumption, and failed feeding attempts (Houde, 1977; MacKenzie *et al.*, 1990). Edge effects may also affect feeding. In highly restrictive environments, such as

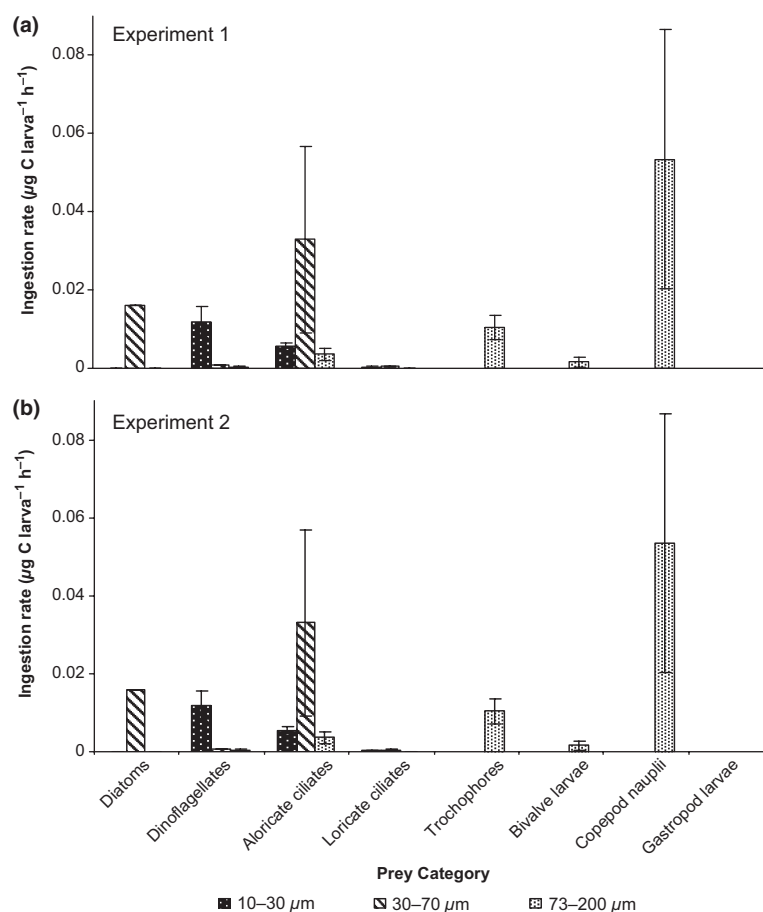


Figure 8. Ingestion rates ($\mu\text{g C larva}^{-1} \text{h}^{-1}$) in experiment 1 (a) and experiment 2 (b) for taxa within size categories in treatments containing 16 herring. Error bars: ± 1 SE.

the 1-L chambers used in this study, larvae may spend up to 50% of the time at the tank wall (Munk and Kiorboe, 1985). Lighting in laboratory conditions can also be a source of variation, as irradiance affects the ability of the larvae to see their prey (Browman *et al.*, 2005). In addition, ingestion rates in the field can be affected by other factors, such as prey distribution and composition, turbulence, and other environmental conditions (Fiksen and Folkvord, 1999). Therefore, the ingestion rates calculated in this study may not be directly applicable to field conditions (Rothschild and Osborn, 1988) and we therefore recommend further controlled laboratory experiments that mimic natural conditions.

It is also important to note the differences between direct and indirect feeding studies. The methods used in our study indirectly determined feeding by enumerating prey items removed from the seawater rather than directly in the guts of the larvae. Indirect methods of feeding detection assume that prey growth is consistent with and without predators (Lessard *et al.*, 1996). Our prey growth rates indicated that

there was no significant difference between growth in control jars and treatment jars, and we chose short incubation times to obviate any potential predator effects. Trophic interactions in the protist community are complex (Pierce and Turner, 1992) and the protists in our study could have been preyed upon by predators other than the herring larvae. But, again, the controls would have accounted for any of these dynamics, such that the differences we saw between controls and treatments could only be attributable to the presence of the larval herring.

Larval preconditioning as a source of variation

Although there were clear patterns of feeding by larval herring across our experiments, there was considerable variability in prey selectivity and ingestion rates. Initial prey abundance may influence the resulting variability in clearance and ingestion rates because ingestion generally increases as prey abundance increases, until feeding becomes saturated (MacKenzie *et al.*, 1990). However, initial prey abundance was not significantly different among our experiments

($P = 0.095$), and therefore should not have influenced larval feeding rates. Variability may also occur due to individual differences in the herring larvae, such as age, individual fitness, gape size, and body length. As the larvae used in our experiments were chosen at random from batch cultures, there could have been up to 1 day or 1 mm difference in hatching and length, respectively. Even though the larvae were housed under identical conditions prior to experiments, intra-cohort variability may influence individual feeding success and fitness (Hillgruber and Kloppmann, 2001). Additionally, our experiments conducted in May and June used herring larvae from different spawning stocks, which may have introduced additional variability.

Another potential source of variability in our feeding experiments was preconditioning of the larvae to their prey. Feeding success of larval fish increases if they have been exposed previously to the prey type (Dutton, 1992), and the larvae used in our study were not preconditioned to a natural assemblage of prey. However, the larvae were reared on a mixed diet of *Artemia* nauplii, rotifers, and algae, the movement and escape responses of which are comparable to copepod nauplii, heterotrophic protists, and diatoms (which lack an escape response), respectively, and which therefore should have allowed the larvae to recognize a wide variety of prey items. Nevertheless, preconditioning the larvae to an even more diverse assemblage of prey could have increased feeding and thus the number of significant results.

Implications of protists in the diet of larval fish

Larval fish are often thought to be food limited in nature due to the abundance and distribution of their prey (Buckley and Durbin, 2006), although field evidence of food limitation is equivocal. However, the abundance of heterotrophic protists has been underestimated or not considered in many larval fish diet studies, and thus there may be more food available to larval fish than previously recognized (Figueiredo *et al.*, 2005). Protists are a potentially significant food source for larval fish due to their high abundance, biomass, and growth rates (Pierce and Turner, 1992; Lessard *et al.*, 1996). The available biomass of heterotrophic protists is on the same order of magnitude as that of copepod nauplii, and in some areas have higher growth and turnover rates (Uye *et al.*, 1996; Fukami *et al.*, 1999). Additionally, in some areas there may be a stronger correlation between the spatial distribution of fish larvae and heterotrophic protists than with copepod nauplii (Lasker *et al.*, 1970; Zhang *et al.*, 2002) because many metazoan zooplankton are

patchy in their distribution (as are protists, albeit at potentially different scales) and exhibit strong diel vertical migration (Young *et al.*, 2009).

The consumption of heterotrophic protists by larval fish may be less energetically expensive than the consumption of copepod nauplii, even though the net energy gain from the capture of a nauplius is two orders of magnitude higher than for the capture of a heterotrophic protist (Hunt von Herbing *et al.*, 2001). Additionally, consumption of both loricate and aloricate ciliates may enhance survivability of fish larvae in the absence of larger, more nutritious prey items (Nagano *et al.*, 2000). Larval fish require various highly unsaturated fatty acids, phospholipids, inositol and choline for adequate growth, survival, and metamorphosis (Sargent *et al.*, 1999). Calanoid copepods provide fairly ideal amounts of these nutrients, but it is unclear whether heterotrophic protists alone could provide sufficient amounts for long-term growth and survival. Heterotrophic and mixotrophic dinoflagellates are capable of biochemically upgrading food, producing essential fatty acids and/or sterols which may be absent in bacteria and phytoplankton, whereas marine ciliates only repackage their food and do not add value to it (Klein Breteler *et al.*, 1999). Thus heterotrophic protists could be crucial for larval survival during early stages of development because these larval fish may not be able to fully digest copepods and nauplii (O'Connell, 1981).

Heterotrophic protists provide a link between the microbial food web and metazoans, constitute a significant part of the mesoplankton diet, and are the primary grazers of phytoplankton and bacteria (e.g., Porter *et al.*, 1985; Pierce and Turner, 1992; Rollwagen-Bollens *et al.*, 2006, 2011). Our study demonstrates a direct link between protists and larval Pacific herring. This traditionally unrecognized trophic pathway may create a more efficient transfer of energy from lower trophic levels to planktivorous fishes, potentially increasing larval fish growth and survival. This may ultimately influence recruitment success because a small decrease in mortality can significantly affect year-class strength (Houde, 1987).

CONCLUSIONS

This study examined feeding dynamics of larval Pacific herring on natural assemblages of prey with the inclusion of soft-bodied heterotrophic protists. We found that when presented with a natural assemblage of prey, larval herring consumed aloricate ciliates, loricate ciliates, and dinoflagellates, even in the presence of metazoan prey. Herring larvae showed strong

selection for bivalve larvae and 73–200 μm prey, but protists were generally selected for at rates comparable to metazoans. We also found that ingestion of carbon biomass from protists was of the same magnitude as, and in some cases exceeded, ingestion of metazoan carbon. These results suggest that protists may be more important in the diet of larval fish than previously recognized, and confirm that there is a direct trophic link between protists and larval fish, which may have implications for larval growth, survival and subsequent recruitment success.

ACKNOWLEDGEMENTS

We would like to thank P. Hershberger and J. Gregg of the USGS Marrowstone Marine Field Station for their support and the use of larval herring and laboratory space. We also thank A. Gibson and C. Ross for help with plankton identification, B. Tissot for statistical assistance, and B. Tissot and S. Henderson for comments on earlier drafts of this manuscript. Support for this research was provided by Washington State University Vancouver and an NSF GK-12 Fellowship (NSF Award # 07-42561 to G.R.B and S.M.B.).

REFERENCES

- Arthur, D.K. (1976) Food and feeding of larvae of three fishes occurring in the California Current, *Sardinops sagax*, *Engraulis mordax*, and *Trachurus symmetricus*. *Fish. Bull. US* **74**:517–530.
- Bollens, S.M. (1988) A model of predatory impact of larval marine fish on the population dynamics of their zooplankton prey. *J. Plankton Res.* **10**:887–906.
- Bollens, S.M. and Sanders, A. (2004) Ecology of larval Pacific herring *Clupea pallasii* in the San Francisco Estuary: seasonal and interannual abundance, distribution, diet and condition. *Am. Fish. Soc. Symp.* **36**:15–35.
- Browman, H.I., Skiftesvik, A.B. and Kuhn, P. (2005) The relationship between ultraviolet and polarized light and growth rate in the early larval stages of turbot (*Scophthalmus maximus*), Atlantic cod (*Gadus morhua*) and Atlantic herring (*Clupea harengus*) reared in intensive culture conditions. *Aquaculture* **256**:296–301.
- Buckley, L.J. and Durbin, E.G. (2006) Seasonal and inter-annual trends in the zooplankton prey and growth rate of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) larvae on Georges Bank. *Deep-Sea Res. II* **53**:2758–2770.
- Checkley, D.M. (1982) Selective Feeding by Atlantic Herring (*Clupea harengus*) Larvae on Zooplankton in Natural Assemblages. *Mar. Ecol. Prog. Ser.* **9**:245–253.
- Chimura, M., Watanabe, Y., Okouchi, H., Shirafuji, N. and Kawamura, T. (2009) Hatch period-dependent early growth and survival of Pacific herring *Clupea pallasii* in Miyako Bay, Japan. *J. Fish Biol.* **74**:604–620.
- Confer, J.L. and Moore, M.V. (1987) Interpreting selectivity indices calculated from field data or conditions of prey replacement. *Can. J. Fish. Aquat. Sci.* **44**:1529–1533.
- Cushing, D.H. (1975) *Marine Ecology and Fisheries*. Cambridge: University Press.
- Dutton, P. (1992) Effects of experience on feeding success by larval white seabass *Atractoscion nobilis*. *J. Fish Biol.* **41**:765–773.
- Figueiredo, G.M., Nash, R.D.M. and Montagnes, D.J.S. (2005) The role of the generally unrecognized microprey source as food for larval fish in the Irish Sea. *J. Mar. Biol.* **148**:395–404.
- Figueiredo, G.M., Nash, R.D.M. and Montagnes, D.J.S. (2007) Do protozoa contribute significantly to the diet of larval fish in the Irish Sea? *J. Mar. Biol. Assoc. UK* **87**:843–850.
- Fiksen, O. and Folkvord, A. (1999) Modelling growth and ingestion process in herring *Clupea harengus* larvae. *Mar. Ecol. Prog. Ser.* **184**:273–289.
- Frost, B.W. (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* **6**:805–815.
- Fukami, K., Watanabe, A., Fujita, S., Yamaoka, K. and Nishijima, T. (1999) Predation on naked heterotrophic protist microzooplankton by fish larvae. *Mar. Ecol. Prog. Ser.* **185**:285–291.
- Gifford, S.M., Rollwagen-Bollens, G.C. and Bollens, S.M. (2007) Mesozooplankton omnivory in the upper San Francisco estuary. *Mar. Ecol. Prog. Ser.* **348**:33–46.
- Hardy, A.C. (1924) The herring in relation to its animate environment. Part I. The food and feeding habits of the herring with special reference to the east coast of England. *Fish. Invest. Lond.* (2) **7**:1–53.
- Hershberger, P., Elder, N., Wittouck, J., Stick, K. and Kocan, R.M. (2005) Abnormalities in larvae from the once-largest Pacific herring population in Washington State result primarily from factors independent of spawning location. *Am. Fish. Soc. Symp.* **134**:326–337.
- Hillebrand, H.H., Dürselen, C.C., Kirschtel, D., Pollinger, U. and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* **35**:403–424.
- Hillgruber, N. and Kloppmann, M. (2001) Small-scale patterns in distribution and feeding of Atlantic mackerel (*Scomber scombrus* L.) larvae in the Celtic Sea with special regard to intra-cohort cannibalism. *Helgol. Mar. Res.* **55**:135–149.
- Hjort, J. (1914) Fluctuations in the great fisheries in Northern Europe viewed in light of biological research. *Rapp. Congr. CIESM* **20**:1–228.
- Houde, E.D. (1977) Effects of stocking density and food density on survival, growth, and yield of laboratory-reared larvae of red sea bream, *Archosargus rhomboidalis* (L.) (Sparidae). *J. Fish Biol.* **7**:115–127.
- Houde, E.D. (1987) Fish early life dynamics and recruitment variability. *Am. Fish. Soc. Symp.* **2**:17–29.
- Houde, E.D. (1989) Comparative growth, mortality and energetics of marine fish larvae: temperature and implied latitudinal effects. *Fish. Bull. US* **87**:471–495.
- Hunt von Herbing, I. and Gallager, S.M. (2000) Foraging behavior in early Atlantic cod larvae (*Gadus morhua*) feeding on a heterotrophic protist (*Balanion* sp.) and a copepod nauplius (*Pseudodiaptomus* sp.). *Mar. Biol.* **136**:591–602.
- Hunt von Herbing, I., Gallager, S.M. and Halteman, W. (2001) Metabolic costs of pursuit and attack in early larval Atlantic cod. *Mar. Ecol. Prog. Ser.* **216**:201–212.
- Klein Breteler, W.C.M., Schogt, N., Baas, M., Schouten, S. and Kraay, G.W. (1999) Trophic upgrading of food quality by heterotrophic protists enhancing copepod growth: role of essential lipids. *Mar. Biol.* **135**:191–198.

- Lasker, R. (1975) Field criteria for the survival of anchovy larvae: the relation between inshore chlorophyll maximum layers and successful first feeding. *Fish. Bull. US* **73**:453–462.
- Lasker, R., Feder, H.M., Theilacker, G.H. and May, R.C. (1970) Feeding, growth and survival of *Engraulis mordax* larvae reared in the laboratory. *Mar. Biol.* **5**:345–353.
- Last, J.M. (1978a) The food of four species of pleuronectiform larvae in the eastern English Channel and southern North Sea. *Mar. Biol.* **45**:359–368.
- Last, J.M. (1978b) The food of three species of gadoid larvae in the eastern English Channel and southern North Sea. *Mar. Biol.* **48**:377–386.
- Lechowicz, M.J. (1982) The sampling characteristics of electivity indices. *Oecologia* **52**:22–30.
- Leggett, W.C. and Deblois, E. (1994) Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? *Neth. J. Sea Res.* **32**:119–134.
- Lessard, E.J., Martin, M.P. and Montagnes, D.J.S. (1996) A new method for live-staining protists with DAPI and its application as a tracer of ingestion by walleye Pollock (*Theragra chalcogramma* (Passas)) larvae. *J. Exp. Mar. Biol. Ecol.* **204**:43–57.
- Lynn, D.H. and Montagnes, D.J.S. (1991) Global production of heterotrophic marine planktonic ciliates. In: *Protozoa and Their Role in Marine Processes*. P.C. Reid & P.H. Burkil (eds) Berlin: Springer, pp. 281–307.
- MacKenzie, B.R., Leggett, W.C. and Peters, R.H. (1990) Estimating larval fish ingestion rates: can laboratory values be reliably extrapolated to the wild? *Mar. Ecol. Prog. Ser.* **67**:209–225.
- Marin, V., Huntley, M.E. and Frost, B. (1986) Measuring feeding rates of pelagic herbivores: analysis of experimental design methods. *Mar. Biol.* **93**:49–58.
- Menden-Deuer, S. and Lessard, E.J. (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol. Oceanogr.* **45**:569–579.
- Munk, P. and Kiorboe, T. (1985) Feeding behaviour and swimming activity of larval herring (*Clupea harengus*) in relation to density of copepod nauplii. *Mar. Ecol. Prog. Ser.* **24**:15–21.
- Nagano, N., Iwatsuki, Y., Kamiyama, T. and Nakata, H. (2000) Effects of marine ciliates on survivability of the first-feeding larval surgeonfish, *Paracanthurus hepatus*: laboratory rearing experiments. *Hydrobiologia* **432**:149–157.
- Nakagawa, Y., Eguchi, M. and Miyashita, S. (2007) Pacific bluefin tuna, *Thunnus orientalis*, larvae utilize energy and nutrients of microbial loop. *Aquaculture* **267**:83–93.
- O'Connell, C.P. (1981) Development of organ systems in the Northern Anchovy, *Engraulis mordax*, and other teleosts. *Am. Zool.* **21**:429–446.
- Ohman, M.D., Theilacker, G.H. and Kaupp, S.E. (1991) Immunochemical detection of predation on ciliates protists by larvae of the northern anchovy (*Engraulis mordax*). *Biol. Bull.* **181**:500–504.
- Pepin, P. and Dower, J.F. (2007) Variability in the trophic position of larval fish in a coastal pelagic ecosystem base on stable isotopic analysis. *J. Plankton Res.* **29**:727–737.
- Pierce, R.W. and Turner, J.T. (1992) Ecology of planktonic ciliates in marine food webs. *Rev. Aquat. Sci.* **6**:139–181.
- Porter, K.G., Sherr, E.B., Sherr, B.F., Pace, M. and Sanders, R.W. (1985) Protozoa in planktonic food webs. *J. Protozool.* **32**:409–415.
- Reitan, K.I., Natyik, C.M. and Vadstein, O. (1998) Drinking rate, uptake of bacteria and microalgae in turbot larvae. *J. Fish Biol.* **53**:1145–1154.
- Rollwagen-Bollens, G.C. and Penry, D.L. (2003) Feeding dynamics of *Acartia* spp. Copepods in a large, temperate estuary (San Francisco Bay, CA). *Mar. Ecol. Prog. Ser.* **257**:139–158.
- Rollwagen-Bollens, G.C., Bollens, S.M. and Penry, D.L. (2006) Vertical distribution of micro- and nanoplankton in the San Francisco Estuary in relation to hydrography and predators. *Aquat. Microb. Ecol.* **44**:143–163.
- Rollwagen-Bollens, G.C., Gifford, S.M. and Bollens, S.M. (2011) The role of protistan microzooplankton in the upper San Francisco Estuary planktonic food web: source or sink? *Estuar Coast* **34**:1026–1038.
- Rossi, S., Sabates, A., Latasa, M. and Reyes, E. (2006) Lipid biomarkers and trophic linkages between phytoplankton, zooplankton and anchovy (*Engraulis encrasicolus*) larvae in the NE Mediterranean. *J. Plankton Res.* **28**:551–562.
- Rothschild, B.J. and Osborn, T.R. (1988) Small-scale turbulence and plankton contact rates. *J. Plankton Res.* **10**:465–474.
- Sargent, J., McEvoy, L., Estevez, A. et al. (1999) Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* **179**:217–229.
- Spittler, P., Brenning, U. and Arlt, G. (1990) Heterotrophic protists – the first food of larval herring (*Clupea harengus* L.)? *Int. Rev. Gesamt. Hydrobiol.* **75**:597–603.
- Stoecker, D.K. and Capuzzo, J.M. (1990) Predation on protozoa: its importance to zooplankton. *J. Plankton Res.* **12**:891–908.
- Uye, S., Nagano, N. and Tamaki, H. (1996) Geographical and seasonal variations in abundance, biomass and estimated production rates of microzooplankton in the Inland Sea of Japan. *J. Oceanogr.* **52**:689–703.
- Vanderploeg, H.A. and Scavia, D. (1979a) Calculation and use of selectivity coefficients of feeding: Zooplankton grazing. *Ecol. Modell.* **7**:135–149.
- Vanderploeg, H.A. and Scavia, D. (1979b) Two electivity indices for feeding with special reference to zooplankton grazing. *J. Fish. Res. Bd Can.* **36**:362–365.
- Vdovin, A.N. and Chernoiivanova, L.A. (2006) Long-term dynamics of certain parameters of Pacific herring *Clupea pallasi* (Clupeidae) in Peter the Great Bay. *J. Ichthyol.* **46**:50–57.
- Yamaguchi, A., Watanabe, Y., Ishidi, H. et al. (2005) Biomass and chemical composition of net-plankton down to greater depths (0–5800 m) in the western North Pacific Ocean. *Deep-Sea Res.* **52**:341–353.
- Young, K.V., Dower, J.F. and Pepin, P. (2009) A hierarchical analysis of the spatial distribution of larval fish prey. *J. Plankton Res.* **31**:687–700.
- Zhang, W., Xu, K., Wan, R. et al. (2002) Spatial distribution of ciliates, copepod nauplii and eggs, *Engraulis japonicus* post-larvae and microzooplankton herbivorous activity in the Yellow Sea, China. *Aquat. Microb. Ecol.* **27**:249–259.