Environ Biol Fish (2006) 76:303–315
DOI 10.1007/s10641-006-9034-3

ORIGINAL PAPER

Ontogeny of feeding in two native and one alien fish species from the Murray-Darling Basin, Australia

Zeb D. Tonkin · Paul Humphries · Peter A. Pridmore

Received: 5 August 2005 / Accepted: 21 March 2006 / Published online: 3 June 2006

Abstract Investigations into the feeding of the early stages of fishes can provide insights into processes influencing recruitment. In this study, we examined ontogenetic changes in morphology and feeding behaviour of two native Australian freshwater species, Murray cod, *Maccullochella peelii peelii*, and golden perch, *Macquaria ambigua*, and the alien species, common carp, *Cyprinus carpio*. Murray cod free embryos are large and well developed at the onset of feeding, whereas the other two species begin exogenous feeding much younger and are smaller and less-developed. Carp commence exogenous feeding 3 days earlier than golden perch, and show more advanced development of the eyes and ingestive apparatus. We conducted feeding experiments, presenting larvae of the three species with a standardised prey mix (comprising equal numbers of small calanoid copepods, large calanoid copepods, small *Daphnia*, and large *Daphnia*). Larvae of most tested ages and species showed a preference for mid-sized prey (300–500 μm wide). This was true even when their gapes substantially exceeded the largest prey offered. *Daphnia* were consumed more than similar-sized copepods. The results of this study suggest that survival through their larval period will be threatened in all three species if catchable prey < 500 μm in width are not available throughout such time. They also suggest that interspecific competition for prey may occur, especially when larvae are very young. The precocious development of structures involved in feeding and the extended transition from endogenous to exogenous feeding of early carp larvae are likely to have contributed to the success of this species since its introduction to Australia.

Keywords Larval development · Prey preference · Recruitment

Z. D. Tonkin (✉) · P. A. Pridmore
Department of Environmental Management and Ecology, La Trobe University, P.O. Box 821, Wodonga, Victoria 3689, Australia
e-mail: Zeb.Tonkin@dse.vic.gov.au

P. Humphries
Cooperative Research Centre for Freshwater Ecology, Murray-Darling Freshwater Research Centre, P.O. Box 921, NSW 2640, Australia and Department of Biological Sciences, Monash University, Clayton, Victoria 3168, Australia

Present address: Z. D. Tonkin, Freshwater Ecology, Arthur Rylah Institute for Environmental Research, Department of Sustainability and Environment, P.O. Box 137, Heidelberg, Victoria 3084, Australia

Present address: P. Humphries, The Johnstone Centre, School of Environmental and Information Sciences, Charles Sturt University, P.O. Box 789, Albury, NSW 2640, Australia

Introduction

Prey availability is a major factor governing survival and growth during the early life history of fishes (e.g. Frank and Leggett 1982, 1986; Arumugam and Geddes 1987; Cushing 1990; Wainwright and Richard 1995). It directly affects mortality through starvation and can also act indirectly through its effects on growth and condition, and the subsequent vulnerability of young fish to predation and/or disease (Chick and Van Den Avyle 2000). The interval during which free embryos switch from endogenous to exogenous feeding and the timing of this switch relative to the occurrence of high densities of appropriately-sized prey, have been hypothesised as being critical in determining survival and subsequent year class strength (Frank and Leggett 1982; Cushing 1990).

The relationship between the young stages of fishes and their prey is, however, far from simple and is inherently dynamic. The early life history of fishes is a period of rapid development, marked by substantial, sometimes dramatic, changes in structure, physiology and behaviour (Fuiman and Higgs 1997; Balon 1999). These developmental changes strongly influence a young fish's ability to detect, pursue, capture, successfully handle and ingest prey (Wainwright 1988). Thus, size- and age-related differences in structure, physiology and behaviour are usually associated with changes in foraging ability and, in turn, with differential exploitation of food resources (Appelbaum and Schemmel 1983; Livingston 1987; Munk 1992; Luczkovich et al. 1995; McCormick 1998; Sanchez-Velasco 1998). Accordingly, before we can assess the role that prey availability plays in the survival of the young stages of fishes, we must understand how ontogenetic changes affect their access to prey.

In the Murray-Darling Basin (MDB) of Australia, poor survival of the early life history stages, rather than lack of spawning, has been postulated as one of the causes of the decline in range and abundance of many species of native fishes (Gehrke 1991; Koehn 1995; Mallen-Cooper et al. 1995; Humphries and Lake 2000; Humphries et al. 2002). Yet little is known about the ecology of fishes during this critical period; especially their modes of foraging and prey preferences. Most of our knowledge on the food of larvae of Murray-Darling fishes has been from hatchery studies (e.g. Arumugam and Geddes 1992; Gehrke 1992; Rowland 1996), although King (2002) has recently described the diets of the larvae of several species from a river in the south of the basin.

In this study, we investigated aspects of the foraging of two native and one alien MDB fish species, with the aim of (1) documenting morphological changes in larvae of the three species which might be expected to affect feeding ability (i.e. changes in structures associated with sensory function, ingestion and locomotion), which occur over the period from the onset of exogenous feeding to the early juvenile stage; (2) measuring the effect of age, size and species on the capacity of larvae to consume prey of a variety of sizes when such prey are presented in consistent proportions.

Study species

The percichthyids Murray cod, *Maccullochella peelii peelii* (Mitchell), and golden perch, *Macquaria ambigua* (Richardson), are both native to the basin and highly prized as both table fish and sport fish for recreational anglers. Both species have undergone dramatic reductions in distribution and abundance over the past century[1] (Keenen et al. 1995; Koehn 1995). Spawning in these two species occurs in spring and summer. Murray cod produce demersal, adhesive eggs that number in the thousands to tens-of-thousands, with large well-developed free embryos hatching around 10 days after spawning (Humphries et al. 1999). In contrast, golden perch lay pelagic eggs that number in the hundreds-of-thousands and hatching takes as little as 24 h, yielding free embryos that are small and underdeveloped (Humphries et al. 1999).

The third study species, the Boolarra strain of carp, *Cyprinus carpio* L., has prospered since its introduction to northern Victoria during the

[1] Murray Darling Basin Commission, 2002. http://www.mdbc.gov.au/naturalresources/basin_stats/statistics.htm. Accessed 19 February 2002

mid-1960s, so that the species as a whole is now recognised as noxious[1] (Roberts and Ebner 1996). Carp spawning peaks during spring–early summer (Humphries et al. 2002; Smith and Walker 2004) with a single female producing up to 1.54 million eggs (Sivakumaran et al. 2003). The demersal adhesive eggs are laid on submerged macrophytes or detritus (Roberts and Ebner 1996) and hatch in as little as 2 days (Tonkin, personal observation). The presence of such large numbers of larvae of this exotic species also gives rise to the issue of potential of competition, both within and between native and exotic species, during times of limiting prey.

Materials and methods

Collection of free embryos and morphological description

We obtained free embryos of Murray cod and golden perch from the Inland Fisheries Research Station at Narrandera, NSW, following the spawning of captive broodstock. Free embryos of Murray cod were collected as a batch from a single spawning prior to the commencement of exogenous feeding. Known-age free embryos and larvae of golden perch were obtained from rearing ponds approximately 24 h before required. Common carp were collected as eggs from amongst macrophytes in the waters of Lake Hume, NSW (36°01′ S 147°03′ E) during the months of September, October and November. These were subsequently incubated in housing aquaria and monitored daily to determine the date of hatching.

We transported eggs and free embryos of the three study species to the laboratory in insulated, aerated containers. The free embryos and larvae of Murray cod and carp were housed in 70 l aquaria under ambient temperature (approx. 16–24°C) and light conditions. Other than during feeding trials, fish were fed live zooplankton (*Daphnia* and copepods) every 2 days. Golden perch, which we obtained in batches of around 50 individuals, were kept overnight in 4 l aquaria located in a controlled temperature room at 22°C, and used in experiments the following day.

We used a stereo microscope and eyepiece graticule to measure gape, total length (TL) and eye-diameter of 10 individuals of each of 3 or 4 age groups of each species to the nearest 0.01 mm. Gape was measured as the external distance between the two corners of the mouth when shut (Arumugam and Geddes 1987) and TL was measured as the distance between the tip of the snout and the hind-most point of the caudal fin with the body-axis straight, on freshly euthanased individuals. Eye diameter was measured, after preservation in 70% ethanol, as the maximum lateral distance across the eye.

As the morphometric data were found to be normally distributed they were used untransformed in all analyses. Regressions were performed to establish the relationships of age, gape and eye diameter to TL, using the statistical package SPSS[2] and the models that gave the best fit are reported below.

To determine the level of ossification for each free embryo or larval stage, we took photographs of 3–4 fresh specimens of each species at each of three or four age classes. The specimens photographed were ones that approximated the mean dimensions of their age class and which exhibited minimal damage to external features. These specimens were subsequently cleared and stained with alizarin red S, using methods described in Hildebrand (1968). The photographs were used in conjunction with the preserved specimens to assess maturation in a range of other morphological features, including head and body shape, size and ossification of fins, and eye pigmentation.

Feeding trials

Feeding trials were undertaken with the aim of detecting any ontogenetic changes in diet. Calanoid copepods and the cladoceran *Daphnia carinata* were used as prey in these trials. We chose these prey types because they represented large and small prey relative to the gapes of the fish investigated, because they were available throughout the period that trials were run, and

[2] SPSS Inc., 1999. SPSS for Windows (version 10). SPSS Inc., Chicago, USA

because they are abundant in the environments where the fish species concerned typically occur.

We collected zooplankters using a 100 μm mesh net from two local sites. One of the sites was dominated by calanoid copepods, whereas the other was dominated by *Daphnia carinata*. Zooplankters from each site were taken back to the laboratory and put through 2–5 sieves, to grade each prey type into two size classes. Grading produced four prey groups belonging to three size classes, which were characterised according to two morphological parameters (Table 1):

(i) Mean width—measured as the maximum cross-sectional diameter. The mean width of 10 randomly selected individuals was determined by measurement under a microscope using an eyepiece graticule.
(ii) Mean dry weight—calculated as the mean weight of 100 dried zooplankters. Three foil weighing vessels for each prey group were dried to constant weight in a 60°C oven. One hundred zooplankters were then counted into each vessel and put back in the oven for 24 h, after which they were re-weighed and dried to constant weight.

Note: A broad size range was included in group 3, because large *Daphnia* were very prone to damage during the sieving process, which often caused high mortality. We prepared solutions containing each prey group by hand-counting, using a black teaspoon, viewed under a stereo microscope. Prey were dropped on to the spoon with a pipette, examined for condition, and if healthy, added to the vial. This method was time consuming, but gave accurate counts and avoided the inclusion of dead or damaged individuals.

Table 1 Mean mass and width of prey groups (±1 SE)

Prey group	Size class (μm)	Mass per 100 individuals (μg)	Width (μm)
1	100–300	580 ± 120	195 ± 8
2a	300–500	720 ± 120	334 ± 23
2b	300–500	900 ± 115	396 ± 11
3	500–1,000	1,800 ± 218	720 ± 46

Prey group 1 = small calanoid copepods; prey group 2a = large calanoid copepods; prey group 2b = small *Daphnia carinata*; prey group 3 = large *Daphnia carinata*

Prior to running a feeding trial, we half-filled six 50 ml vials with conditioned water. Prey were then added to the vials so each contained 100 of prey group 1 (small calanoids), 100 of prey group 2 (50 large calanoids and 50 small *Daphnia*) and 100 of prey group 3 (large *Daphnia*). Thus, there were approximately 300 zooplankters in each vial and, consequently, equal proportions of the three prey sizes.

Feeding trials were run using different developmental stages (see Serafini and Humphries 2004) and ages of Murray cod, golden perch and carp, ranging from first feed (Muray cod and golden perch only—age at first feed determined from Rowland, 1992; Arumugam and Geddes 1987) to metalarvae (Table 2).

Six 2 l vessels containing 1 l of conditioned water, were equipped with air stones and left in a constant temperature room (22°C) for a minimum of 24 h. Approximately 5 min before the addition of larvae, the air stones were removed. Lighting was kept constant throughout all experiments by an overhead fluorescent light.

Groups of 10 fish larvae of a predetermined age and species were placed in each of three of the vessels and left to acclimatise for 10 min. The other three vessels served as controls and contained no fish. After 10 min, a volume of water containing the 300 live zooplankters (100 of each size class) was added to the first vessel. Similar numbers were added to the other five vessels at 2-min intervals (to allow for handling at completion of a trial). The volume of water containing the prey was adjusted to 1 l to give a prey density of 300 zooplankton l^{-1} for each vessel.

Larvae were allowed to feed for 1 h, after which the entire contents of each vessel (including fish larvae) were immediately concentrated in a smaller vessel and euthanased using benzocaine. Larvae and zooplankters were then separated from the solution using a small 100 μm sieve and preserved in 70% ethanol for later analysis. Remaining zooplankton in each replicate were separated from the preserving solution using a 100 μm sieve, emptied into small perspex sorting trays and examined under a stereo microscope. The numbers of remaining (non-consumed)

Table 2 Morphological characteristics of each age group (including approximate stages; [PL]Postlarvae, [Pr]Protolarvae, [F]Flexion, [PF]Postflexion, [M]Metalarvae) of larvae of the three study species

Species	Age (days post-hatch)	TL ± 1 SE (mm)	Gape ± 1 SE (mm)	Eye diameter ± 1 SE (mm), description and position	Yolk sac	Fins	Flexion of notochord	Ossification			
								Cranium	Max and pre-max	Vert	Fin rays
Murray cod $n = 30$	11^{PL*}	12.62 ± 0.09	1.67 ± 0.02	0.91 ± 0.01, pig. ant.	Yes	D, C, A, P	Yes	Inc.	Yes	Yes	D, C, A, P
	15^{PL*}	13.35 ± 0.08	1.75 ± 0.02	0.95 ± 0.01, more post.	No	D, C, A, P, Pel	Yes	Inc.	Yes	Yes	D, C, A, P
	19^{PL*}	13.80 ± 0.07	1.87 ± 0.01	0.99 ± 0.12 more post.	No	D, C, A, P, Pel	Yes	Yes	Yes	Yes	D, C, A, P, Pel
Common carp $n = 30$	2^{Pr}	6.74 ± 0.11	0.59 ± 0.00	0.55 ± 0.01, pig., ant.	Yes	P	No	No	Inc.	Inc.	None
	9^{F*}	8.61 ± 0.21	0.83 ± 0.05	0.56 ± 0.01, ant.	Yes	P, C	Yes	Inc.	Inc.	Most	C
	15^{PF*}	9.26 ± 0.18	0.94 ± 0.02	0.65 ± 0.02, more post.	No	P, C, D, A	Yes	Inc.	Yes	Yes	C
	22^{M*}	12.51 ± 0.39	1.23 ± 0.05	0.88 ± 0.04	No	P, C, D, A, Pel	Yes	Inc.	Yes	Yes	P, C, D, A, Pel
Golden perch $n = 40$	6^{Pr*}	5.03 ± 0.02	0.46 ± 0.01	0.34 ± 0.01, pig., ant.	Yes	P	No	No	No	No	None
	11^{F}	6.41 ± 0.18	0.84 ± 0.03	0.48 ± 0.02, pig.	No	P	Yes	Inc.	Yes	Inc.	P, C
	13^{PF*}	10.38 ± 0.24	1.52 ± 0.09	0.76 ± 0.02, more pos.	No	P, C, D, A, Pel	Yes	Inc.	Yes	Yes	P, C, D, A, Pel
	19^{M*}	11.66 ± 0.19	1.84 ± 0.06	0.85 ± 0.02	No	P, C, D, A, Pel	Yes	Yes	Yes	Yes	P, C, D, A, Pel

*Identifies ages of larvae used in feeding trials; TL—total length. pig.—pigmented; ant.—anterior; post.—posterior; inc.—incomplete; D—dorsal; C—caudal; A—anal; P—pectoral; Pel—pelvic

members of each prey group were recorded, with any uncertainties of assignment by size clarified by measuring the particular prey organism with an eyepiece graticule.

Feeding trial data were used to calculate prey selectivity and consumption values for each species separately. Consumption rates were assessed for each tested age group of each species in two ways; as number of individual zooplankters ingested within 1 h (initial number of prey from each group minus the remaining number) and as the total mass of prey consumed within 1 h (calculated by multiplying the number of each prey group consumed, by the mean individual prey dry-weights determined earlier in the prey analysis). Consumption rates of both were then analysed for each species using a one-way analysis of variance (ANOVA) using each of the two measures of prey consumption as the dependent variables, and larval age as the factor. Post-hoc comparisons were then undertaken using the Tukey HSD test.

Prey selectivity was assessed using Manly's alpha (α_i) for variable prey populations (Krebs 1989). This index was considered appropriate, because of the decreasing proportions of prey over the duration of the feeding interval. Calculations were made using the formula:

$$\alpha_i = \log p_i \Big/ \sum_{j=i}^{m} p_j$$

where p_i, p_j = proportion of prey i or j remaining at the end of the experiment ($i = 1, 2, 3,...,m$; $j = 1, 2, 3,...,m$) = e_i/n_i, e_i = number of prey type i remaining uneaten at end of experiment, n_i = initial number of prey type i in experiment, m = number of prey types.

For each trial, the mean number of zooplankters of each prey group found preserved in the controls was used as the initial prey number (n_i). This provided a better estimate of initial prey number than the figure generated during prey preparation (up to 10% underestimation for smaller prey classes). Alpha values obtained from the selectivity index were subsequently analysed using a two-way ANOVA to test selectivity for significance using 'Age' and 'Prey size' as the independent variables.

Results

Ontogenetic change in morphology

Murray cod free embryos at 11 days post-hatch (age at first-feed) were approximately 12 mm in TL, had pigmented eyes, a flexed notochord, advanced ossification of the skeleton, and all fins (except pelvics) present with rays ossified. Nevertheless, these fish still retained considerable stores of yolk (Table 2). By 15 days, their eyes were in a more posterior position, ossification was almost complete and pelvic fins were now present. At 19 days, growth was continuing, but there was little change in gross morphology; although ossification was complete.

Common carp free embryos at 2 days post-hatch (age at first-feed) were approximately 6.5 mm in TL, had pigmented eyes, yolk sacs, unflexed notochords, were elongated in shape and only showed signs of ossification in visceral arches and maxilla (Table 2). Only pectoral fins were present. By 9 days, flexion had taken place and ossification had advanced so that most of the vertebrae and the newly formed caudal fin was ossified. All yolk had been consumed by 15 days and the larvae were becoming streamlined. At this age ossification of the skeleton was advanced (including all vertebrae) and (other than pelvics) all fins were present. By 22 days old, carp had taken on a juvenile appearance, acquired all fins, and ossification was complete.

Golden perch larvae at 6 days post-hatch (age at first-feed) were approximately 5 mm in TL and relatively undeveloped. They had pigmented eyes, limited yolk sacs, their notochords had not flexed, there were no signs of ossification and only pectoral fins were present (Table 2). Ossification had begun by 11 days, and, at this age, the yolk had been consumed and notochord had flexed. The caudal fin was present and its rays were ossified. At 13 days old, larvae had grown to approximately 10 mm TL, all fins were present with ossification advanced. Little change had taken place in morphology of golden perch between 13 and 19 days, apart from the fact that ossification was completed.

Age, gape and eye diameter varied with total length in the early post-hatch of all three species (Fig. 1). It is evident from this figure that the rate of increase in total length was more even in carp and Murray cod than in golden perch over the age ranges studied. Golden perch showed the greatest rate of increase in total length between days 11 and 13.

Smaller (younger) specimens of carp and golden perch had similar gapes (Fig. 1), but their gapes diverged with increasing total length (and age) due to gape increasing more rapidly in golden perch than in carp. Murray cod had an intermediate gape; at least over the length (age) range we examined.

Eye diameter was similar in smaller specimens of carp and golden perch, but diverged with increasing total length (and age) due to more rapid growth of the eyeball in carp than in golden perch (Fig. 1). Murray cod had eye diameters similar to golden perch of the same length: at least over the length (age) range we examined. For all study species, Length was far superior to Age as a predictor of Gape and Eye diameter. Accordingly, all data are regressed with TL only.

Feeding trials

One-way ANOVA tests indicated that both the number and mass of plankton consumed differed significantly with age in all three species (Table 3, Fig. 2). Post-hoc comparisons indicated that the oldest ages of larvae of all three species consumed greater numbers and mass of prey than their younger age classes of larvae (Fig. 2). Murray cod at 11 days of age consumed a significantly greater number, but not mass, of prey than 15 day-old larvae (Fig. 2a).

Prey selection by Murray cod larvae did not change significantly with age ($P > 0.05$), and prey from the 300–500 μm size class and prey from group 2b (small *Daphnia*) were significantly preferred over prey from other size classes and prey groups ($P < 0.001$), (Table 4, Fig. 3a). There was no significant interaction between larval age and prey size ($P > 0.05$).

Prey selection by carp did not change significantly with age ($P > 0.05$), but two-way ANOVA tests showed that there was an interaction between the factors larval age and prey size and larval age and prey group for prey selection by

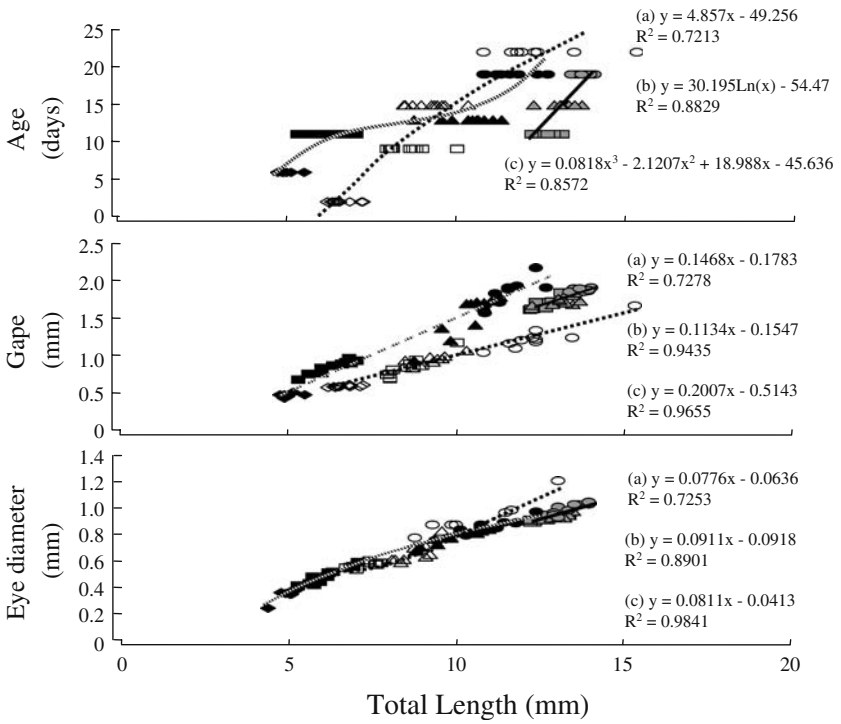

Fig. 1 Relationship between length and age; gape; and eye diameter (including regression equations) for (**a**) Murray cod (grey); (**b**) carp (white), and (**c**) golden perch (black). Equivalent shapes identify larvae of similar age both within and between species

carp ($P < 0.001$) (Table 4), most probably due to the increased selection for prey of group 1 by 22 day-old larvae (Fig. 3b). All tested ages of carp larvae consumed little prey from the 500–1,000 μm size class, preferring prey from the smaller size classes and group 2b (Fig. 3b).

As with the other two study species, prey selection by golden perch larvae did not change significantly with age ($P > 0.05$), and prey size and prey group were significant factors in prey selection ($P < 0.001$, $P < 0.01$, respectively) (Table 4, Fig. 3c). The youngest age group of larvae preferred the smallest size class of prey, whilst the other ages of larvae showed a strong preference for prey size 2, and group 2b (Fig. 3c). Two-way ANOVA test of prey selection by golden perch showed no evidence of an interaction between larval age and prey size ($P > 0.05$), but an interaction between the factors larval age and prey group ($P < 0.001$) was very evident (Table 4).

Table 3 *F*-values, significance levels and degrees of freedom for one-way ANOVA testing of consumed zooplankton number and biomass relative to age for Murray cod, carp and golden perch larvae

Species	df	Number consumed	Mass consumed (μg)
Murray cod	2, 24	7.108*	18.64***
Carp	2, 24	30.248***	26.651***
Golden perch	2, 24	278.982***	365.951***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Discussion

Morphological changes in the visual and locomotor systems

Development of the visual field is important for prey selectivity, because it influences a fish's ability to recognise absolute prey size; indeed, prey location capacity has been shown in some species to increase exponentially with fish length (Wazenbock and Schiemer 1989). Given that the resolving power of a fish's eye is directly proportional to the diameter of the lens (Zaunreiter et al. 1991), and assuming a positive relationship between eye diameter and lens diameter (Fernald 1985), substantial increases in visual acuity are

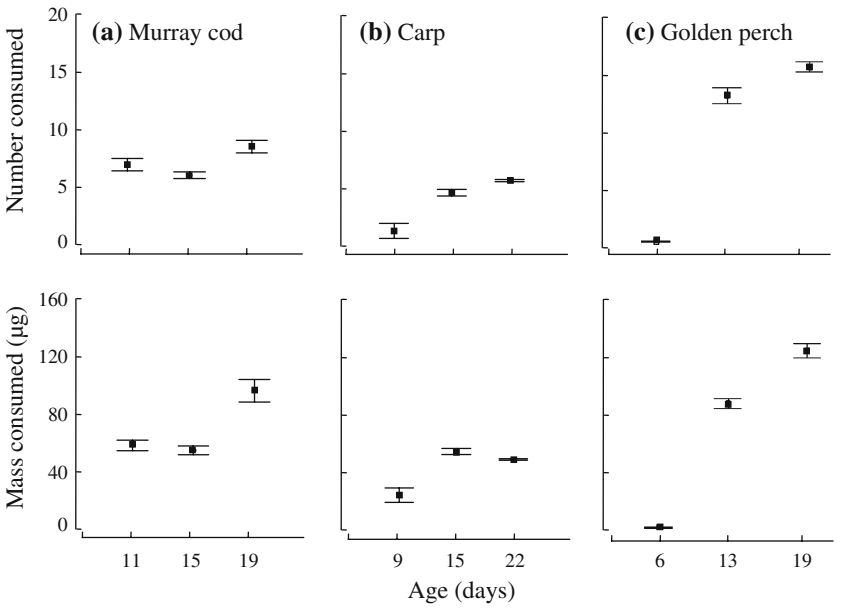

Fig. 2 Mean number and dry weight (±1 SE) of prey consumed per individual during a 1 h feeding trial by the larvae of (**a**) Murray cod, (**b**) carp and (**c**) golden perch of indicated ages

likely during early growth in Murray cod, carp and golden perch.

The typical pattern of swimming of young fish requires high flexibility over the whole of the body and tail (Osse 1990), suggesting that extensive development of bone may be disadvantageous during early stages. Thus the distinct lack of ossification of the fins and vertebral column we observed in the early developmental stages of golden perch and carp may reflect the functional demands of the locomotor system. The larger Murray cod larvae had well ossified fins and vertebral column at all ages examined. This is not surprising, given that this species uses a swimming mode quite unlike that used by similar-aged individuals of the other species (Tonkin, personal observation).

Pectoral fins were evident in the earliest hatchlings of carp and golden perch examined in the present study. These fins serve to generate forces that reduce yawing (sideways) movements of the head, which are associated with swimming in young free embryos and larvae, and so the presence of these fins ought to allow for improved aiming during feeding (Osse 1990). Similarly, early ossification of the posterior soft-rayed portion of the dorsal fin of Murray cod and golden perch, probably improves manoeuvrability (Alexander 1967) which suggests that manoeuvrability may be more important than protective spines to the survival of early stages of these two species.

Table 4 *F*-values and significance levels for two-way ANOVA testing of prey selection with respect to larval ages and prey sizes and with respect to larval ages and prey groups

	df	Murray cod	Carp	Golden perch
Prey size				
Age	2, 18	0.000	0.000	0.000
Prey size	2, 18	67.831***	45.674***	18.091***
Age × prey size	4, 18	1.822	10.650***	1.521
Prey group				
Age	2, 24	0.000	0.000	0.000
Prey group	3, 24	89.168***	9.246***	6.434**
Age × prey group	6, 24	1.044	2.790*	2.717*

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Morphological changes in the prey ingestion apparatus

The three species examined use a suction mode of feeding, involving rapid expansion of the buccal cavity (Tonkin, personal observations), with all having the ability to protrude their upper jaw during feeding by extending the premaxillae. We made no attempt to analyse the mechanisms

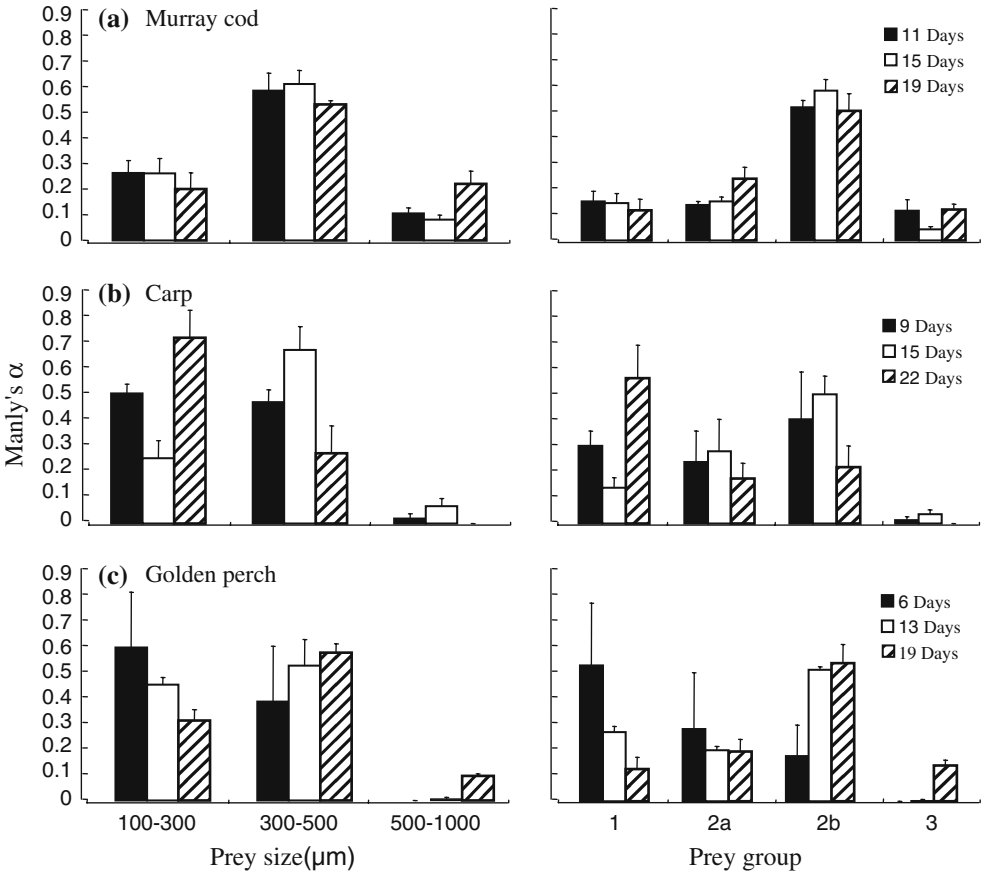

Fig. 3 Prey size and prey group selection using Manly's $\alpha \pm 1$ SE values during a 1 h feeding trial by the larvae of (**a**) Murray cod, (**b**) carp and (**c**) golden perch of indicated ages

involved in these actions. Nevertheless, changes in gape and the timing of ossification of the major structures associated with prey ingestion were noted and provide a basis for both structural and functional comparisons among species.

We found that gape was directly proportional to body length in all specimens of Murray cod, golden perch and carp examined. This is in accord with findings from most larval fish investigated to date (e.g. Arumugam and Geddes 1987; Schael et al. 1991; Mehner et al. 1998; Sanchez-Velasco 1998). If gape-limited feeding is assumed for these species, then the change in gape with length suggests that the potential for inter-specific competition for food should be greatest at early larval stages and become progressively less as they grow.

The proportionately large size of the jaws of Murray cod larvae, and their fully ossified condition from at least 11 days of age, suggest that they have effective suction and perhaps jaw protrusion at this age. Carp larvae begin exogenous feeding at 2 days of age and we observed that the lower jaw and visceral arches of carp larvae of this age were substantially ossified; although, neither the maxillae nor premaxillae appeared to be fully ossified at this age, so that perhaps such larvae had not yet developed the capacity to feed with a protrusible jaw. However, Drost and Van Den Boogaart (1986) noted that another part of the buccal-suction apparatus, the opercular valve, matures very early in carp. Thus, carp seem to be remarkably precocious in the development of structures associated with suction feeding. With respect to this, it is relevant to note that the increase in buccal volume per unit fresh weight in the early life history of carp is three times higher than in the adults of other fish such

as perch (Drost et al. 1988). In contrast, we could detect no sign of ossification of the feeding apparatus of golden perch until 11 days of age.

An interesting strategy employed by the early post-hatch stages of carp is the source of endogenous nutrition (the yolk sac) remaining in evidence for up to a week after the commencement of exogenous feeding. Murray cod larvae do use a similar strategy, commencing feeding between 9 and 11 days and having complete yolk absorption by 15 days of age (Rowland 1992). This however, also involves producing far fewer eggs yielding large-well developed embryos. In contrast, golden perch, which like carp also produce large numbers of eggs, yield free embryos that have a transition from endogenous to exogenous feeding limited to around 2 days (Rowland 1996).

One of the consequences of a more extended transition from entirely endogenous to entirely exogenous feeding is that the young fish concerned are likely to be better able to survive situations in which exogenous food sources are temporarily unavailable. Thus the longer transition evident in the larvae of carp and Murray cod suggests that they have greater capacity to survive temporary shortages of prey than golden perch larvae.

Feeding trials

In our experiments, we found that age had a significant influence on total prey consumption by Murray cod, carp and golden perch larvae. Similarly, Arumugam and Geddes (1987) found that the daily food consumption of larval golden perch was directly proportional to their standard length. A rise in food consumption with age is expected for a variety of reasons. Such a rise is likely to reflect increasing experience in capturing and handling prey (Pankhurst et al. 1991; Fuiman and Higgs 1997). Although, ontogenetic changes in larval structures, such as eyes, teeth, bones and fins, should also promote increased prey capture. Of course, the increase in consumption with age also reflects the increased appetite arising from the physiological demand of a growing animal for increased energy.

Fish larvae generally select prey sizes well below the limits imposed by their gape (e.g. Mills et al. 1986; Schael et al. 1991; Munk 1992; Bremigan and Stein 1994; Mehner et al. 1998; Krebs and Turingan 2003) and our results are consistent with this. Despite possessing gapes that would have allowed the ingestion of all available prey, Murray cod and carp larvae, at all three tested ages, and golden perch, at two of the three tested ages, chose the same mid-sized prey. The only exception to the selection of prey well below gape-limit occurred with 6 day-old golden perch, which consumed prey approaching their maximum gape. The larvae concerned were barely past first-feed and the smaller prey sizes offered to them were at the upper limit of their gape. Nevertheless, such ingestion of prey in the upper limits imposed by gape has been reported for first feeding larvae of this species (Arumugam and Geddes 1992; Gerhke 1992; Rowland 1996) and for blue whiting *Micromesistius poutassou* (González-Quirós and Anadón 2001).

The strong preference of small specimens of *Daphnia* (prey group 2b) over similar-sized calanoids (prey of group 2a) by all ages and in all species studied during our experiments confirms observations made on golden perch larvae by Arumugam and Geddes (1992) and indicates that the same is true of some other Murray-Darling Basin fishes. Our results also support the work of McLaren and Avendaño (1995) who have shown that size alone does not determine prey choice, but that characteristics related to prey taxon are important in prey selection. Videographic recordings of the escape behaviour of prey used in the present study show that specimens of *Daphnia* have a slower escape speed than similar-sized copepods (Tonkin unpublished data). This is consistent with the finding of Furnass (1979) that the fry of perch, *Perca fluviatilis*, expend more effort capturing adult calanoid copepods than in capturing *Daphnia* of similar size. However, other energetic or dietary considerations may also be involved. For example, Furnass (1979) found the energy content of *Daphnia* is 2.4 times that of copepods. Additionally, it is possible that fish larvae can detect cladocerans more readily than other prey types (Mayer and Wahl 1997). Thus, the preference for small specimens of *Daphnia* over similar-sized calanoids we observed may reflect ease of prey capture, greater net gain in

energy per unit effort expended in capture, greater detectability of *Daphnia* or some combination of all three.

The preference of the 9 and 15 day-old carp larvae for small *Daphnia* is in agreement with Vilizzi (1998) who found cladocerans to be the main food component (both percentage composition and number of items per standard length) of carp larvae collected in the wild. Reasons for the increase in consumption of the smallest prey size by 22 day-old carp larvae is unclear although they may simply have been using prey of which were less evident to their younger conspecifics. Wazenbock and Schiemer (1989) found prey detection in cyprinid larvae to increase exponentially with fish size. The basis of this change in prey awareness is unclear, but it may be that larger larvae are able to detect smaller prey more easily.

Conclusions

Humphries and Lake (2000) have advanced the hypothesis that poor recruitment may underlie the decline evident in populations of several freshwater fishes native to the Murray-Darling Basin. Conversely, it is self-evident that the expanding populations of some exotic species in the same regions reflect successful recruitment.

One obvious inference from the results obtained from our study is that the survival of the larvae of Murray cod, golden perch and common carp will be in doubt if they do not encounter abundant prey < 500 μm in width, especially during the first 3 weeks after hatching. Whether foraging is improved by inundation of the floodplain, or the opposite (Humphries et al. 1999), it is obvious that prey must be available that are of a suitable size, are catchable and able to be handled and, that the prey must occur within a microhabitat suitable to the larvae involved.

Despite not being directly addressed in the present study, a question of potential competition between larvae must be asked. With larvae of all tested ages and species showing a preference for midsized prey and the co-occurrence of larvae of all three species in the wild (Humphries et al. 2002) it is clear that this may be an important issue when prey is limiting, particularly between carp and native fish. Furthermore, given the relatively extended transition from entirely endogenous to entirely exogenous feeding seen in carp larvae, it seems likely that they would better survive short periods of prey shortage than the larvae of at least some native fishes in which this transition occurs more rapidly.

Acknowledgments We thank Steve Thurstan from the Narrandera Inland Fisheries Research station for provision of native fish and advice on fish husbandry. Catherine Meathrel of La Trobe University provided advice on statistical analysis. Staff of the Murray-Darling Freshwater Research Centre provided access to temperature control facilities and aquarium supplies. This study was carried out under the La Trobe University Animal Ethics Committee, permit number AEC01/37(W)/V1.

References

Alexander RM (1967) Functional design in fishes. Hutchinson & Co. Ltd, London, 160 pp

Appelbaum S, Schemmel C (1983) Demersal sense organs and their significance in the feeding behaviour of the common sole *Solea vulgaris*. Marine Ecol Progr Ser 13:29–36

Arumugam PT, Geddes MC (1987) Feeding and growth of golden perch larvae and fry (*Macquaria ambigua*). Trans Roy Soc South Aust 111:59–65

Arumugam PT, Geddes MC (1992) Selectivity of microcrustacean zooplankton by golden perch (*Macquaria ambigua*) larvae and fry in laboratory studies. Trans Roy Soc South Aust 116:29–32

Balon EK (1999) Alternative ways to become a juvenile or a definitive phenotype (and on some persisting linguistic offenses). Environ Biol Fishes 56:17–38

Bremigan MT, Stein RA (1994) Gape-dependent larval foraging and zooplankton size: implications for fish recruitment across systems. Can J Fish Aquat Sci 51:913–922

Chick JH, Van Den Avyle MJ (2000) Effects of feeding ration on larval swimming speed and responsiveness to predator attacks: implications for cohort survival. Can J Fish Aquat Sci 57:106–115

Cushing DH (1990) Plankton production and year class strength in fish populations: an update of the match/mismatch hypothesis. Adv Marine Biol 26:249–293

Drost MR, Van Den Boogaart JGM (1986) The energetics of feeding strikes in larval carp, *Cyprinus carpio*. J Fish Biol 29:371–379

Drost MR, Muller M, Osse JWM (1988) A quantitative hydrodynamical model of suction feeding in larval fishes: the role of frictional forces. Proc Roy Soc Lond B 234:263–281

Fernald RD (1985) Growth of the teleost eye: novel solutions to complex constraints. Environ Biol Fish 13:113–123

Frank KT, Leggett WT (1982) Environmental regulation of growth rate, efficiency, and swimming performance in larval capelin (*Mallotus villosus*) and its application to the match/mismatch hypothesis. Can J Fish Aquat Sci 39:691–699

Frank KT, Leggett WT (1986) Effect of prey abundance and size on the growth and survival of larval fish: an experimental study employing large volume enclosures. Marine Ecol Progr Ser 34:11–22

Fuiman LA, Higgs DM (1997) Ontogeny, growth and the recruitment process. In: Chambers RC, Trippel EA (eds) Early life history and recruitment in fish populations. Chapman & Hall, London, pp 225–249

Furnass TI (1979) Laboratory experiments on prey selection by perch fry (*Perca fluviatilis*). Freshwater Biol 9:33–43

Gehrke PC (1991) Avoidance of inundated floodplain habitat by larvae of golden perch (*Macquaria ambigua* Richardson): influence of water quality or food distribution? Aust J Marine Freshwater Res 42:707–719

Gehrke PC (1992) Diel abundance, migration and feeding of fish larvae (Eleotridae) in a floodplain billabong. J Fish Biol 40:695–707

González-Quirós R, Anadón R (2001) Diet breadth variability in larval blue whiting as a response to plankton size structure. J Fish Biol 59:1111–1125

Hildebrand M (1968). Anatomical preparations. University of California Press, Berkley, CA, USA, 100 pp

Humphries P, Lake PS (2000) Fish larvae and the management of regulated rivers. Regulat Rivers 16:421–432

Humphries P, King AJ, Koehn JD (1999) Fish, flows and floodplains: links between freshwater fishes and their environment in the Murray-Darling River system, Australia. Environ Biol Fish 56:129–151

Humphries P, Serafini LG, King A (2002) River regulation and fish larvae: variation through space and time. Freshwater Biol 47:1307–1331

Keenan C, Watts R, Serafini L (1995) Population genetics of golden perch, silver perch and eel-tailed catfish within the Murray-Darling Basin. Murray Darling Basin Commission, 1995 Riverine Environment Research Forum, pp 17–26

King, AJ (2002) Recruitment ecology of fish in floodplain rivers of the Southern Murray-Darling Basin, Australia. Ph.D. thesis. Monash University, Australia

Koehn J (1995) Habitats and movements of freshwater fish in the Murray-Darling Basin. Murray Darling Basin Commission, 1995. Riverine Environment Research Forum, pp 27–32

Krebs CJ (1989) Ecological methodology. HarperCollins Publishers, New York, NY, USA, 654 pp

Krebs JM, Turingan RG (2003) Intraspecific variation in Gape–prey size relationships and feeding success during early ontogeny in red drum, *Sciaenops ocellatus*. Environ Biol Fish 66:75–84

Livingston ME (1987) Morphological and sensory specialization of five New Zealand flatfish species, in relation to feeding behaviour. J Fish Biol 31:775–795

Luczkovich JL, Norton SF, Gilmore RG (1995) The influence of oral anatomy on prey selection during the ontogeny of two percoid fishes, *Langodon rhomboides* and *Centropomus undecimalis*. Environ Biol Fish 44:79–95

Mallen-Cooper M, Stuart I, Hides-Pearson F, Harris J (1995) Fish migration in the River Murray and assessment of the Torrumbarry fishway. Murray Darling Basin Commission, 1995. Riverine Environment Research Forum, pp 33–37

Mayer CM, Wahl DH (1997) The relationship between prey selectivity and growth and survival in a larval fish. Can J Fish Aquat Sci 54:1504–1512

McCormick MI (1998) Ontogeny of diet shift by a macrocarnivorous fish, *Cheilodactylus spectabilis:* relationship between feeding mechanics, microhabitat selection and growth. Marine Biol 132:9–20

McLaren IA, Avendaño P (1995) Prey field and diet of larval cod on Western Bank, Scotian Shelf. Can J Fish Aquat Sci 52:448–463

Mehner T, Plewa M, Hulsmann S, Worischka S (1998) Gape-size dependent feeding of age-0 perch (*Perca fluviatilis*) and age-0 zander (*Stizostedion lucioperca*) on *Daphnia galeata*. Arch Hydrobiol 142: 191–207

Mills EL, Confer JL, Kretchmer DW (1986) Zooplankton selection by young yellow perch: the influence of light, prey density, and predator size. Trans Am Fish Soc 115:716–725

Munk P (1992) Foraging behaviour and prey size spectra of larval herring *Clupea harengus*. Marine Ecol Progr Ser 80:149–158

Osse JWM (1990) Form changes in fish larvae in relation to changing demands of function. Netherlands J Zool 40:362–385

Pankhurst PM, Montgomery JC, Pankhurst NW (1991) Growth, development and behaviour of artificially reared larval *Pagrus auratus* (Bloch & Schneider, 1801) (Sparidae). Aust J Marine Freshwater Res 42:391–398

Roberts J, Ebner B (1996) The issue of carp in Australia. Murray Darling Basin Commission, 1996. Riverine Environment Research Forum, pp 7–12

Rowland SJ (1992) Diet and feeding of Murray Cod (*Maccullachella peelii*) larvae. Proc Linn Soc NSW 113:193–201

Rowland SJ (1996) Development of techniques for the large-scale rearing of the larvae of the Australian freshwater fish golden perch, *Macquaria ambigua* (Richardson, 1845). Marine Freshwater Res 47:233–242

Sanchez-Velasco L, (1998) Diet composition and feeding habits of fish larvae of two co-occurring species (Pisces: Callionymidae and Bothidae) in the North-western Mediterranean. ICES J Marine Sci 55:299–308

Schael DM, Rudstam LG, Post JR (1991) Gape limitation and prey selection in larval yellow perch (*Perca flavescens*), freshwater drum (*Aplodinotus grunniens*), and black crappie (*Pomoxis nigromaculatus*). Can J Fish Aquat Sci 48:1919–1925

Serafini LG, Humphries P (2004) Preliminary guide to the identification of larvae of fish, with a bibliography of their studies, from the Murray-Darling Basin.

Cooperative Research Centre for Freshwater Ecology. Identification & Ecology Guide No. 48. Albury and Clayton, Vic., Australia

Sivakumaran KP, Brown P, Stoessel D, Giles A (2003) Maturation and reproductive biology of female wild carp, *Cyprinus carpio*, in Victoria, Australia. Environ Biol Fish 68:321–332

Smith BB, Walker KF (2004) Spawning dynamics of carp (*Cyprinus carpio* L.) in the River Murray, South Australia, shown by macroscopic and histological staging of gonads. J Fish Biol 64:1–19

Vilizzi L (1998) Observations on ontogenetic shifts in the diet of 0 + carp, *Cyprinus carpio*, from the River Murray, Australia. Folia Zool 47:225–229

Wainwright PC (1988) Morphology and ecology: functional basis of feeding constraints in Caribbean labrid fishes. Ecology 69:635–645

Wainwright PC, Richard BA (1995) Predicting patterns of prey use from morphology of fishes. Environ Biol Fish 44:97–113

Wanzenbock J, Schiemer F (1989) Prey detection in cyprinids during early development. Can J Fish Aquat Sci 46:995–1001

Zaunrieter M, Junger H, Kotrschal K (1991) Retinal morphology of cyprinid fishes: a quantitative histological study of ontogenetic changes and interspecific variation. Vision Res 31:383–394