

# Feeding of a larval clinid fish in a microtidal estuary from southern Chile

Alimentación de larvas de un pez clínido en un estuario micromareal del sur de Chile

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**Resumen.**- Es ampliamente reconocida la utilización de estuarios como zonas de crianza para peces e invertebrados, pero el éxito en la explotación de los recursos variará entre las especies residentes del estuario y las oportunistas. Se estudió la distribución y dieta de larvas de *Myxodes viridis* (Pisces, Clinidae) en 2 localidades del estuario de Bahía Corral (estuario-arriba y estuario-abajo), uno de los estuarios más grandes del sur de Chile, durante la primavera austral de 2009 a 3 profundidades (superficie, pycnoclina, profundo) durante la fase llenante y vaciante de 4 ciclos mareales. Las larvas fueron recolectadas principalmente en la estación estuario-abajo durante fase vaciante. La incidencia alimentaria también fue mayor en la fase vaciante; las larvas cambiaron de omnivoría a carnivoría a medida que se desarrollaban, depredando principalmente sobre nauplii y copepoditos calanoideos. Aunque el éxito alimentario fue similar para cada estado de desarrollo en la columna de agua, la dieta de larvas en preflexión y flexión estuvo basada en diferentes ítems presa dentro de los primeros 3-4 m de la columna de agua (superficie y pycnoclina) con respecto de aguas más profundas (7-10 m, cerca del fondo). Esto sugiere que la pycnoclina pudiera afectar la composición de la dieta de larvas pequeñas de *M. viridis*, pero no el tamaño, número o volumen de presas ingeridas. Además, ya que el forrajeo está basado predominantemente en diferentes estados de desarrollo de copépodos, las larvas que emergen durante vaciante pueden incrementar la advección fuera del estuario hacia zonas costeras cercanas, indicando que esta especie no es un usuario frecuente del estuario durante su desarrollo larval.

**Palabras clave:** *Myxodes viridis*, ictioplancton, éxito alimentario, Bahía Corral

**Abstract.**- The utilization of estuaries as nursery zones for fish and invertebrates is widely known, but differences in the success of resource exploitation vary between resident and opportunistic estuarine species. The distribution and diet of larval *Myxodes viridis* (Pisces, Clinidae) were studied at 2 locations (up-estuary and down-estuary) in the Corral Bay estuary, one of the largest estuaries in southern Chile, during the austral spring 2009 at 3 depths (surface, pycnocline, deep) during the flood and ebb of 4 tidal cycles. Larvae were collected mostly at the down-estuary station during the ebb phase. Feeding incidence was also greater during the ebb phase; larvae changed from omnivory to carnivory as they developed, preying mainly on nauplii and calanoid copepodites. Although the feeding success was similar for each developmental stage along the water column, diet of preflexion and flexion larvae was different in the first 3-4 m depth (surface and pycnocline) in relation to deeper waters (7-10 m depth, near to the bottom). This suggests that the pycnocline may affect the composition of the diet of small, larval *M. viridis* but not the size, number or volume of ingested prey. Additionally, because foraging is predominantly based on the different life stages of copepods, larvae emerging during the ebb tide may increase the off-estuary advection into nearby shelf waters, indicating that this species is not a user of the estuary during its early development.

**Key words:** *Myxodes viridis*, ichthyoplankton, feeding success, Corral Bay

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## INTRODUCTION

Estuaries represent highly dynamic environments, largely utilized as nursery grounds for fishes and crustaceans around the world (Fox *et al.* 1999, Pardo *et al.* 2011). The definition of a nursery is based on 4 comparative factors:

(1) density, (2) growth, (3) survival, and (4) movement to adult habitats, *i.e.*, contribution to the adult population (Beck *et al.* 2003, Able 2005). Larvae of some species recruit in abundance in particular habitats within the

estuary, accumulate biomass and then migrate to other habitats to breed. Typically, these environments have low predation rates and high food availability (Fox *et al.* 1999, Islam *et al.* 2006), but those relationships are likely to vary between resident (with obligate life history stages in the estuary) and facultative users (estuarine opportunists).

In an estuary where physical conditions change over short temporal and spatial scales, it is plausible that the feeding habits of fish larvae may be modified or affected by the vertical structure of the water column. Estuarine-dependent species can be affected by a major physical feature of the estuary, the estuarine turbidity maximum (ETM), an area where larval fish retention and feeding increases (North & Houde 2001, Islam *et al.* 2006). However, this physical feature does not affect the ecology (feeding and/or growth rates) of non estuarine-dependent coastal fish species (Islam *et al.* 2006). As established by Able (2005), there is a need for more research concerning biotic factors (*e.g.*, competition, predation, etc.) to enhance our understanding of estuarine dependence, particularly in marine fish species.

Recently, studies of mesopelagic species that utilize fjord environments as spawning and nursery grounds have described changes in food items (Landaeta *et al.* 2012) and feeding success (*i.e.*, total prey volume per gut, Landaeta *et al.* 2011) in relation to the surface salinity and vertical stratification, respectively. Additionally, large abundance of larval anchoveta *Engraulis ringens* has been detected associated to strong water column stratification (Bustos *et al.* 2008). However, there is little information about the utilization of estuaries by the early life stages of littoral fish species as feeding grounds (Fox *et al.* 1999, Islam *et al.* 2006).

The family Clinidae consists of relatively small (less than 300 mm SL) benthic fishes, the majority of which inhabit intertidal and shallow subtidal waters (Nelson 2006). The Clinidae contains 3 tribes (Myxodini, Clinini, and Ophiclinini), 20 genera and 71 species. The Myxodini is characterized by ovuliparous oviparity and the lack of an intromittent organ in males. Five genera are contained in the Myxodini: *Myxodes*, *Heterostichus*, *Gibbonsia*, *Ribeiroclinus*, and *Clinitrachus*, from which only *Myxodes* are found along the west coast of South America (Stepien 1992). In Chile, 3 species are identified, *Myxodes viridis* Valenciennes, 1836, *M. cristatus* Valenciennes, 1836, and *M. ornatus* Stephens and Springer, 1973 (Stephens & Springer 1973). *M. viridis* inhabit from

Independencia Bay, Peru to southern Chile, feeding mostly crabs, while juveniles (<75 mm SL) primarily ate harpacticoid copepods, amphipods, gastropod snails, and isopods (Stepien 1990). There is no previous information about the feeding habits of either *Myxodes viridis* larvae or other clinid larvae in Chilean waters.

The Corral Bay estuary (*ca.*, 40°S) is a very dynamic estuary, with several interconnected rivers, mainly the Valdivia and Tornagaleones Rivers, which together occupy a hydrographic basin of 13,135 km<sup>2</sup>. The estuary also has marked saline and thermal stratifications (Pino *et al.* 1994) and a microtidal regime characterized by a salt wedge or partially mixed circulation, depending on high or low levels of precipitation, respectively (Davila *et al.* 2002, Pardo *et al.* 2012). Based on 4 oceanographic cruises carried out in the Corral Bay estuary, we studied the feeding behavior in larval *Myxodes viridis*. One question was addressed: Is the pycnocline capable of accumulating potential prey and increase the feeding success of larval *M. viridis*? The hypothesis that the pycnocline in an estuary may affect the composition of prey items and feeding success of a larval clinid during the austral spring season was tested.

## MATERIALS AND METHODS

### FIELD WORK

Four cruises were carried out onboard an artisan vessel at 2 locations in the Corral Bay estuary (39°52'S, 73°25'W, Fig. 1), one with a greater influence of freshwater (up-estuary, Mancera) and the other with a greater influence of shelf waters (down-estuary, San Carlos), during the austral spring 2009 (November-December). During each cruise and at each location, San Carlos and Mancera, the hydrographic conditions (temperature and salinity) were measured throughout the water column using a YSI multiparameter unit, model 556 MPS, and oblique plankton samples were collected with a WP-2 open-close ring net (60 cm diameter, 300 µm mesh size, equipped with a flow meter to estimate the filtered seawater volume) in the mixed layer (1 m depth), pycnocline (2 to 5 m depth) and deep layer (~ 10 m depth) by 5-10 min. The plankton net filtered 3.6 to 72.7 m<sup>3</sup> (mean ± standard deviation: 33.7 ± 15.2 m<sup>3</sup>), being the surface collections shorter in time than the other depth ranges. All plankton samples were fixed with 4% formaldehyde buffered with sodium borate. Physical and biological sampling was repeated 4 times on each cruise and at each location, 2 during the flood and 2 during the ebb tidal phases of each sampling day.

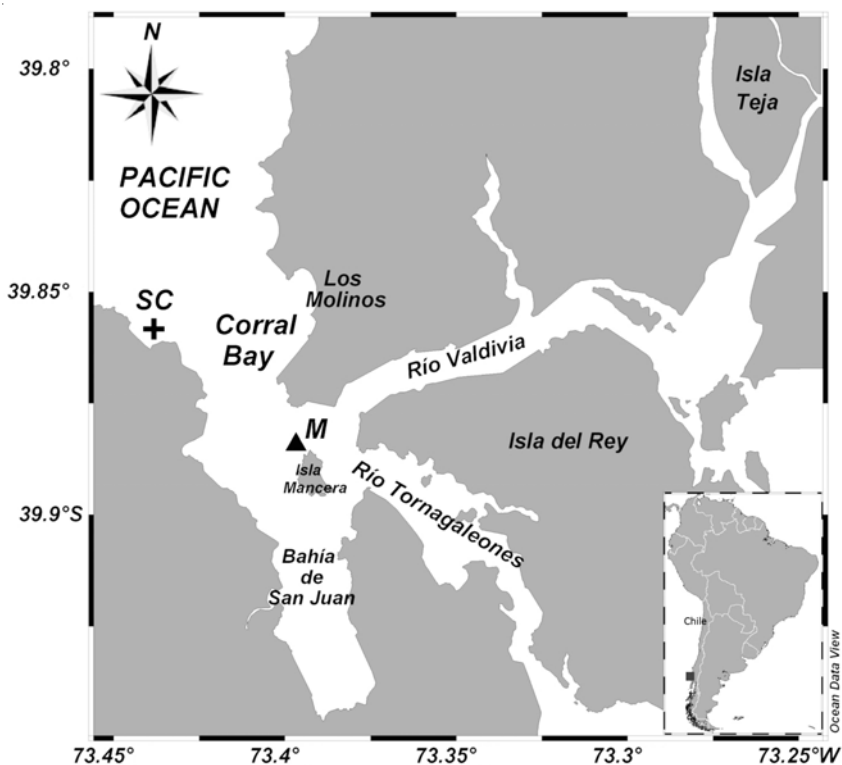


Figure 1. Map of the study area, indicating the up-estuary station (Mancera, black triangle) and the down-estuary station (San Carlos, black cross), at the Corral Bay estuary, southern Chile / Mapa del área de estudio, indicando la estación estuario-arriba (Mancera, triángulo negro) y la estación estuario-abajo (San Carlos, cruz negra), en el estuario de la Bahía Corral, sur de Chile

#### LABORATORY WORK

In the laboratory, all fish larvae from the plankton samples were separated, counted and identified to the lowest taxonomic level. Larval *M. viridis* were identified by using the series method, which consist of positively identifying the largest available larva, or the smallest juvenile, using known adult characters such as fin meristics, and subsequently linking this specimen with progressively smaller specimens by using general morphological and pigment characters until a developmental series is assembled (Neira *et al.* 1998). Postlarval *M. viridis* distinguishes from other Clinidae and Labrisomidae by the number of spines and soft rays in the dorsal fin (D XXIV-XXVI, 6-7), larger preanal length, and persistent punctate melanophores over the gut throughout the larval development (M.F. Landaeta<sup>1</sup>, unpublished data). Abundance was standardized to individuals per 1000 m<sup>3</sup>, taking into account the number of larvae captured and volume of the seawater filtered by the net. Body length (BL, notochord length in preflexion larvae; standard length in flexion and postflexion larvae), upper jaw length (UJL,

from the tip of the snout to the posterior end of the maxilla) and mouth width (MW, in the ventral view, the width between the posterior edges of the maxillae) of all intact larval *M. viridis* ( $n = 291$ ) were measured under an Olympus SZ61 stereomicroscope attached to a camera (Motic moticam 2500, resolution 5.0 MPixel) using Motic Image Plus 2.0 software. The gut of each larva was dissected from the body and opened length-wise with fine needles. Prey items were counted and identified to the lowest possible taxon, except for indigestible prey remains such as setae, under a Motic BA310 microscope. The maximum BL and width of each prey item (maximum prey width, MPW) were measured with the microscope and a Motic moticam 2500 camera (resolution 5.0 MPixel) using the Motic Image Plus 2.0 software. The volume of each prey item was estimated using the three-dimensional shape that most closely resembled the item, following Cass-Calay (2003) and Sun & Liu (2003). The prosome length of copepodite prey was measured because the urosome was often missing.

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## DATA ANALYSIS

All larvae with identifiable prey in their guts were used for the analysis. The relationships between the BL, UJL and MW of the larvae and between the UJL and MPW were determined by linear regression analyses, and comparisons of slopes were performed with a multiple slope test (Zar 1999). Spearman correlations were also calculated.

Larval *M. viridis* were separated into 3 developmental stages, preflexion, flexion and postflexion larvae to assess changes in the feeding incidence (FI) and prey composition. FI was calculated as a percentage of the total number of larvae with gut contents out of the total number of larvae examined (Sassa & Kawaguchi 2004). Comparisons of FI among developmental stages (preflexion, flexion and postflexion), sampling strata (surface, pycnocline, deep), station (down-estuary, San Carlos and up-estuary, Mancera), and tidal phase (flood and ebb), were carried out with contingency tables.

The diet was described using the percent frequency of occurrence (%*F*) of a diet item in larvae with food in their guts, the percent of the total number (%*N*) of diet items that were examined and the percent of volume (%*V*) of each item out of the total volume of prey items. An index of relative importance (IRI) was calculated as follows:  $IRI = (\%N + \%V) \times \%F$ . To readily allow comparisons among prey items, the IRI was the standardized to %IRI for each prey item *i* (Cortés 1997).

To establish the variability in the feeding success of larval *M. viridis* among the vertical strata, 3 measures of feeding success were compared for each developmental stage separately: the number of prey items per gut (PIPG, number), average prey length per gut (PLPG, mm) and total prey volume per gut (TPVG, mm<sup>3</sup>) (Reiss *et al.* 2002). To avoid the effects of location and tides, only larvae collected during the last cruise at the San Carlos station were utilized for this analysis (Table 2). Because the data were not normally distributed (Shapiro-Wilk tests,  $P < 0.001$ ), the measurements of feeding success were compared using the Kruskal-Wallis ANOVA tests and multiple comparisons of mean ranks.

Finally, to establish the differences in the composition of prey among strata, a multivariate approach was utilized separately for each developmental stage. The prey composition (number of prey) of each larvae was  $\log(x+1)$  transformed to enhance the contribution of less abundant prey, and a Bray-Curtis similarity matrix was generated

from these data. Similarities between larvae were graphically represented by nonmetric multidimensional scaling (NMDS) ordination. The degree of correspondence between the distances among points was measured by a stress function. A one-way analysis of similarities (ANOSIM), which is analogous to a univariate analysis of variance, was utilized to determine the differences in prey composition among the strata. Pairwise ANOSIM comparisons were made between groups using 10,000 permutations. When differences were detected, a similarity percentage (SIMPER) analysis was carried out to assess which prey taxa were primarily responsible for the observed differences between groups of samples (*i.e.*, larvae).

## RESULTS

### PHYSICAL SETTINGS

During the 4 tidal cycles studied during austral spring 2009, the hydrographic conditions in the Corral Bay estuary were relatively stable at each sampling station over time (Table 1). Stations located up-estuary (Mancera) were, on average, warmer (~12°C) and fresher (21-24) than waters located down-estuary (San Carlos) (10-12°C and 31-32, respectively) (Table 1). As an example, water column structure of one specific cruise is showed (Fig. 2). The water column structure changed during the tidal phases: during the flood phase, vertical gradients of temperature and salinity were less marked than during the ebb phases at both locations during the last cruise (Fig. 2, Table 1). A halocline occurred at 2-3 m depth at Mancera, but at San Carlos the mixed layer was almost nonexistent, developing a halocline from the surface to 2 m depth during both ebb and flood phases (Fig. 2).

### LARVAL FISH ABUNDANCE

A total of 95 plankton samples were analyzed. From those, 291 larvae of *Myxodes viridis* were identified, corresponding to abundances varying from 16.62 to 4217.46 individuals (ind.) per 1000 m<sup>3</sup> (mean ± sd, 444.24 ± 1014.25 ind. 1000 m<sup>-3</sup>). Larvae were collected at both locations for all samples but almost exclusively during the ebb phase of the tidal cycle (Table 2). Larval abundance was relatively constant at the up-estuary station (Mancera), but it increased through time at San Carlos, reaching the highest values of abundance during mid-December, 2009 (Table 2).

**Table 1. Summary of the physical conditions (temperature and salinity) during the 4 sampling events at 2 stations (Mancera and San Carlos) in the Corral Bay estuary, southern Chile, during the austral spring, 2009** SD = one standar deviation/ Resumen de las condiciones físicas (temperatura y salinidad) durante 4 eventos de muestreo en 2 estaciones (Mancera y San Carlos) en el estuario de la Bahía Corral, sur de Chile, durante la primavera austral de 2009 SD = una desviación estándar

		Temperature (°C)		Salinity	
		Mean ± SD	Range	Mean ± SD	Range
10-11 November	Mancera	11.84 ± 0.79	10.94 - 12.96	20.99 ± 13.44	1.47 - 33.63
	San Carlos	11.25 ± 0.68	10.40 - 13.10	32.02 ± 3.16	23.62 - 34.10
26-27 November	Mancera	13.28 ± 0.67	12.65 - 15.00	22.44 ± 12.27	1.82 - 32.59
	San Carlos	12.85 ± 0.50	12.40 - 14.52	31.59 ± 3.30	19.22 - 33.83
6-7 December	Mancera	12.04 ± 2.10	10.22 - 15.76	24.65 ± 11.65	3.63 - 34.19
	San Carlos	10.73 ± 1.04	9.86 - 14.09	32.96 ± 2.51	23.37 - 34.30
11-12 December	Mancera	12.89 ± 2.36	10.07 - 16.05	23.73 ± 10.31	4.62 - 34.18
	San Carlos	10.90 ± 1.35	9.58 - 14.99	32.12 ± 3.77	21.76 - 34.40

**Table 2. Spatial and temporal variations in the abundance (ind. 1000 m<sup>-3</sup>) of larval *Myxodes viridis* in the Corral Bay estuary, during spring 2009** / Variaciones espaciales y temporales en la abundancia (ind. 1000 m<sup>-3</sup>) de larvas de *Myxodes viridis* en el estuario de la Bahía Corral durante primavera de 2009

		Mancera	San Carlos
10-11 November	Flood	0	0
	Ebb	26.1 ± 7.3	0
26-27 November	Flood	0	0
	Ebb	22.2 ± 7.9	78.4 ± 86.4
6-7 December	Flood	0	100.3 ± 88.4
	Ebb	37.6	226.9 ± 55.9
11-12 December	Flood	0	0
	Ebb	56.4 ± 29.3	1814.0 ± 1724.3

#### BODY AND GAPE SIZE RELATIONSHIPS

Larval *M. viridis* collected during the austral spring 2009 ranged from 3.14 to 14.46 mm BL (mean ± sd, 6.85 ± 1.72 mm). UJL and MW varied between 258.61-1523.53 µm (600.44 ± 211.29 µm) and 137.96-1631.45 µm (512.91 ± 243.37 µm), respectively. Linear regressions were significant ( $P < 0.001$ ) and explained 76.2 and 45.1% of the variability in UJL and MW, respectively (Fig. 3). The jaw length relative to BL varied from 4.95 to 15.96% (8.69 ± 1.62%), and the mouth width relative to BL varied from 2.66 to 16.06% (7.35 ± 2.57%). Additionally, UJL grew faster (107.23 µm UJL mm BL<sup>-1</sup>) than MW (95.02 µm UJL mm BL<sup>-1</sup>) relative to the BL (multiple slope test,  $t = 7.32$ ,  $P < 0.001$ ).

#### FEEDING INCIDENCE

Only well-preserved larval *M. viridis* ( $n = 287$ ) were utilized for the diet analysis. The smallest larva with gut contents was 3.14 mm BL. The feeding incidence (FI) varied significantly among development stages ( $\chi^2 = 9.8$ , d.f. = 2,  $P = 0.007$ ) and between tidal phases ( $\chi^2 = 18.2$ , d.f. = 1,  $P < 0.001$ ). However, there were no significant differences in FI among strata ( $\chi^2 = 0.21$ , d.f. = 2,  $P = 0.898$ ), nor between stations ( $\chi^2 = 1.46$ , d.f. = 1,  $P = 0.226$ ). Consequently, larvae collected during the ebb phase at the flexion stage had the greatest FI.



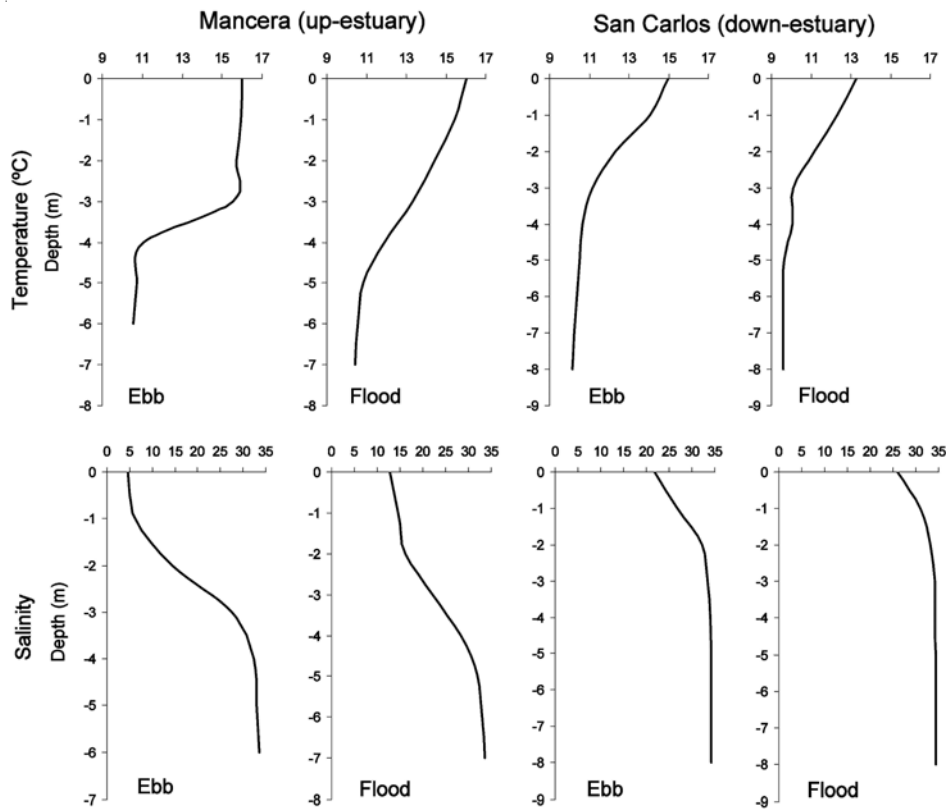


Figure 2. Temperature (°C) and salinity profiles during the ebb and flood tidal phases at Mancera (up-estuary station) and San Carlos (down-estuary station), during 11-12 December 2009 / Perfiles de temperatura (°C) y salinidad durante las fases vaciante y llenante en Mancera (estación estuario-arriba) y San Carlos (estación estuario-abajo) durante el 11-12 de diciembre de 2009

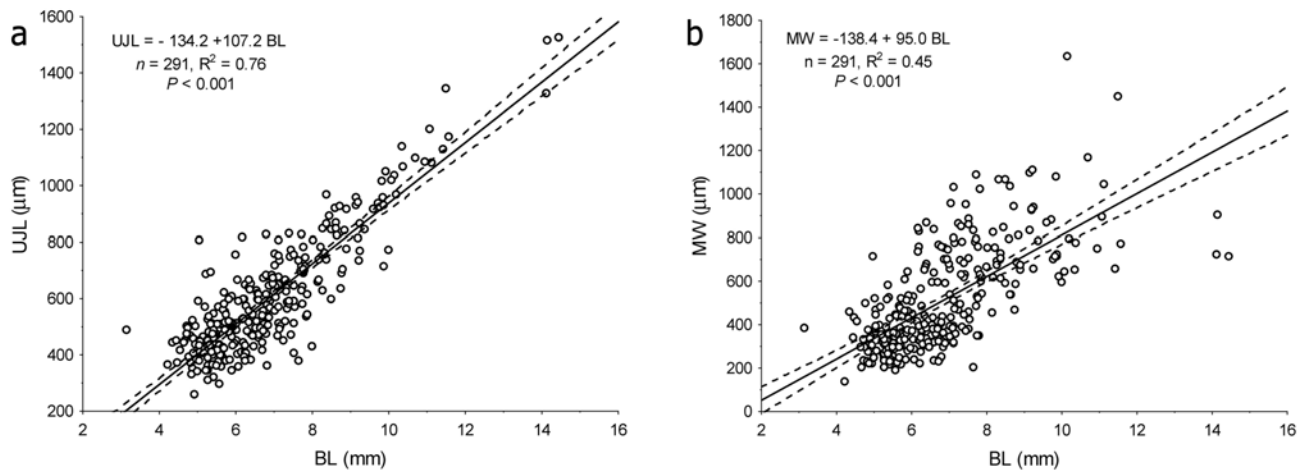


Figure 3. Relationships between body length (BL) and gape size of larval *Myxodes viridis*. a) upper jaw length (UJL), and b) mouth width (MW). Dashed lines correspond to 95% confidence intervals / Relaciones entre longitud corporal (BL) y tamaño del hocico de larvas de *Myxodes viridis*. a) longitud de la mandíbula superior (UJL), y b) ancho del hocico (MW). Las líneas punteadas corresponden a los intervalos de 95% de confianza

### DIET COMPOSITION AND VARIATION DURING LARVAL DEVELOPMENT

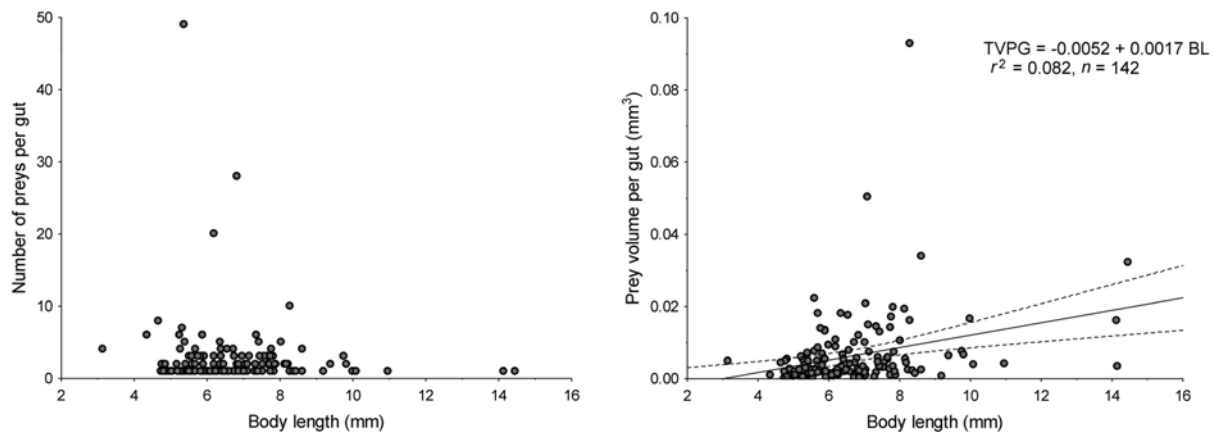
The diet of *M. viridis* was composed of 31 different prey items, phytoplankton and zooplankton prey (Table 3). A change in the diet composition among developmental stages was evident, varying from omnivory to carnivory. Phytoplanktonic prey items were important in smaller larvae (~18%IRI), scarcely found in larvae at the flexion stage (0.36%IRI), and totally absent in postflexion larvae (Table 3). Among zooplankton taxa, all life stages of copepods (eggs, nauplii, metanauplii, copepodite, adults) were ingested in large proportions, ranging from 68.86%IRI (postflexion larvae) to 91.28%IRI (flexion larvae). Larvae in the flexion stage showed the greatest diversity in diet composition ( $S=23$ ) among developmental stages.

### PREY SIZE, NUMBER AND VOLUME OF PREY

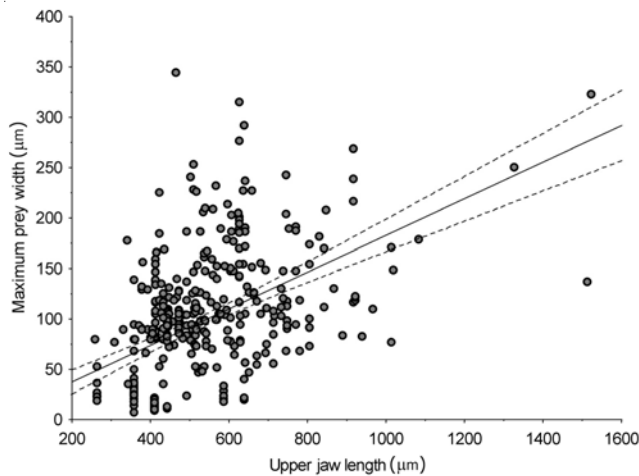
A total of 387 prey were measured. The prey size (*i.e.*, the maximum prey width) of larval *M. viridis* ranged from 7  $\mu\text{m}$  (a diatom) to 344  $\mu\text{m}$  (a balanid nauplius). The number of prey ranged from 0 to 49 ( $2.73 \pm 4.97$  prey items, excluding zeros) and exhibited neither a significant relationship nor a correlation with larval growth (Fig. 4,  $r^2 = 0.007$ ,  $F_{1,140} = 0.97$ ,  $P = 0.325$ ; Spearman  $r = 0.03$ ,  $P > 0.05$ ). Instead, the total volume per gut (TVPG) was positively correlated with the larval size (Spearman  $r = 0.36$ ,  $P < 0.05$ ), and it was weakly explained ( $r^2 = 0.083$ ) by the linear model:  $\text{TVPG} = -0.0052 + 0.0017\text{BL}$  ( $F_{1,140} = 12.62$ ,  $P < 0.01$ ) (Fig. 4). There was a weak but significant positive correlation between the prey size and UJL (Spearman  $r = 0.56$ ,  $P < 0.05$ ). A linear regression model ( $\text{MPW} = 1.14 + 0.18\text{UJL}$ ,  $F_{1,385} = 120.46$ ,  $P < 0.001$ ) explained only 24% of the variability of MPW (Fig. 5).

**Table 3. Composition and variation in prey items of larval *Myxodes viridis* from the Corral Bay estuary, Chile / Composición y variación en los ítems presa de larvas de *Myxodes viridis* del estuario de la Bahía Corral, Chile**

Prey items	Preflexion larvae				Flexion larvae				Postflexion larvae			
	%N	%F	%V	%IRI	%N	%F	%V	%IRI	%N	%F	%V	%IRI
<b>Phytoplankton</b>												
Unidentified microalgae 1	-	-	-	-	0.556	1.190	0.001	0.015	-	-	-	-
Unidentified microalgae 2	-	-	-	-	1.111	1.190	0.001	0.030	-	-	-	-
Unidentified microalgae 3	-	-	-	-	0.556	1.190	0.001	0.015	-	-	-	-
Unidentified diatom	0.532	2.273	0.001	0.035	-	-	-	-	-	-	-	-
<i>Coscinodiscus</i> sp.	24.468	15.909	5.640	14.008	2.778	3.571	0.007	0.222	-	-	-	-
<i>Surirella</i> sp.	28.191	4.545	0.093	3.760	-	-	-	-	-	-	-	-
Cyst	0.532	2.273	0.280	0.054	-	-	-	-	-	-	-	-
Unidentified dinoflagellate	-	-	-	-	1.111	2.381	0.038	0.061	-	-	-	-
<i>Gonyaulax</i> sp.	-	-	-	-	0.556	1.190	0.001	0.015	-	-	-	-
<b>Zooplankton</b>												
Tintiniid	1.596	6.818	3.810	1.078	3.333	5.952	1.806	0.684	-	-	-	-
Bivalve larvae	0.532	2.273	1.253	0.119	-	-	-	-	-	-	-	-
<b>Copepoda</b>												
Copepod eggs	14.362	20.455	8.286	13.547	6.111	7.143	3.390	1.518	-	-	-	-
Nauplii	10.638	31.818	9.695	18.920	30.556	45.238	16.074	47.196	26.316	38.462	13.575	23.920
Metanauplii ( <i>Rhincalampus nasutus</i> )	-	-	-	-	1.111	2.381	0.223	0.071	-	-	-	-
Copepodite	10.638	29.545	32.168	36.986	18.333	29.762	22.882	27.445	42.105	46.154	20.360	44.948
Calanoid copepodite	-	-	-	-	0.556	1.190	2.259	0.075	-	-	-	-
Copepodite ( <i>Acartia tonsa</i> )	-	-	-	-	0.556	1.190	0.378	0.025	-	-	-	-
Copepodite ( <i>Paracalamus indicus</i> )	-	-	-	-	0.556	1.190	0.746	0.035	-	-	-	-
Copepodite ( <i>Rhincalampus nasutus</i> )	-	-	-	-	0.556	1.190	0.211	0.020	-	-	-	-
Copepodite ( <i>Oncaea</i> sp.)	-	-	-	-	0.556	1.190	2.004	0.068	-	-	-	-
Harpacticoid copepodite	0.532	2.273	4.068	0.306	-	-	-	-	-	-	-	-
<i>Paracalamus indicus</i>	0.532	2.273	3.279	0.253	-	-	-	-	-	-	-	-
Nauplii remains	-	-	-	-	3.889	8.333	2.576	1.205	-	-	-	-
Copepodite remains	1.596	4.545	1.705	0.439	12.778	19.048	17.908	13.077	-	-	-	-
Copepod remains	-	-	-	-	2.222	2.381	8.069	0.548	-	-	-	-
Invertebrate eggs	1.064	4.545	0.512	0.209	4.444	9.524	0.989	1.158	15.789	23.077	20.880	13.193
Balanid larvae ( <i>Balanus</i> sp.)	-	-	-	-	-	-	-	-	-	-	-	-
Cypris larvae	-	-	-	-	0.556	1.190	1.169	0.046	10.526	15.385	41.239	12.416
Polychaeta larvae	1.064	4.545	1.286	0.312	-	-	-	-	-	-	-	-
Crustacean remains	0.532	2.273	1.515	0.136	1.111	2.381	1.639	0.146	-	-	-	-
Chitinous remains	3.191	11.364	26.410	9.837	6.111	11.905	17.629	6.323	5.263	38.462	3.946	5.522



**Figure 4. Relationships between number of preys per gut, prey volume per gut and body length of larval *Myxodes viridis*. Dashed lines correspond to 95% confidence intervals / Relaciones entre el número de presas por estómago, volumen de presas por estómago y longitud corporal de larvas de *Myxodes viridis*. Las líneas punteadas corresponden a los intervalos de 95% de confianza**



**Figure 5. Relationships between the gape size (upper jaw length) and prey size (maximum prey width) of larval *Myxodes viridis* captured at the Corral Bay estuary during the austral spring, 2009 / Relaciones entre el tamaño del hocico (longitud de la mandíbula superior) y el tamaño de la presa (ancho máximo de la presa) de larvas de *Myxodes viridis* capturadas en el estuario de la Bahía Corral durante la primavera austral de 2009**

#### FEEDING SUCCESS, DIET COMPOSITION AND WATER COLUMN STRUCTURE

The larval feeding success indicators (PIPG, PLPG and TPVG) did not vary among strata for each developmental stage of larvae (Table 4, Fig. 6). Nevertheless, there was an evident reduction in the number of prey items and an increase in the volume and size of prey throughout the development of larval *Myxodes viridis*.

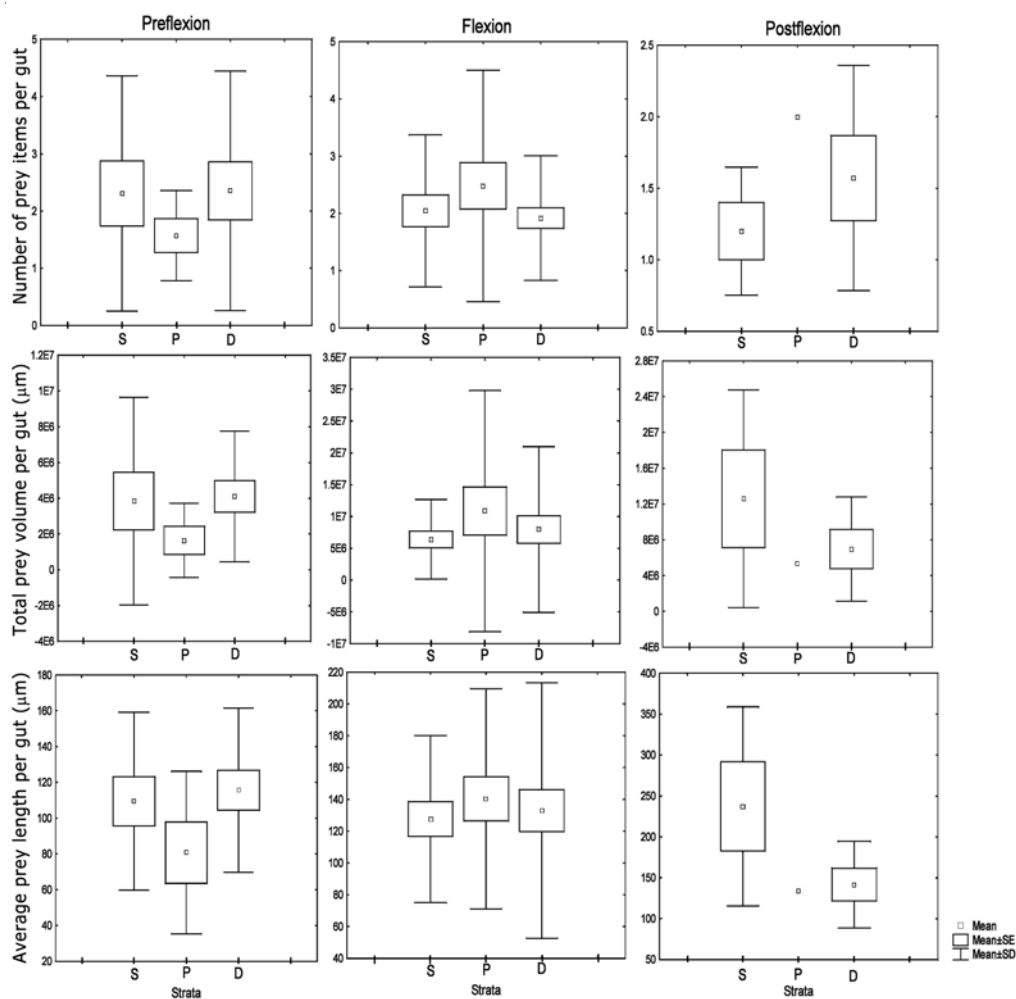
The multivariate analyses indicate that preflexion larvae of *M. viridis* differed in the composition of prey among strata (showed by the NMDS), but in the flexion and postflexion stages, differences among strata were scarce or null (Fig. 7). ANOSIM detected significant differences

(global  $R = 0.084$ ,  $P = 0.014$ ) in the diet of preflexion larvae from surface and deep strata ( $R = 0.096$ ,  $P = 0.016$ ) and between larvae from the pycnocline and deep strata ( $R = 0.128$ ,  $P = 0.041$ ). There were no differences in the diets of preflexion larvae collected at the surface and at the pycnocline ( $R = -0.022$ ,  $P = 0.588$ ). SIMPER analyses indicate that dissimilarities in the diets of larvae collected between surface and deep strata were caused mostly by copepodites (19.50% of contribution), nauplii (17.96%), and copepod eggs (14.32%). Dissimilarities in the feeding habits of larvae collected in the pycnocline and deep strata were due to *Coscinodiscus* sp. (21.62%), copepodites (18.32%) and nauplii (17.47%) prey items. In



**Table 4. Comparison of indices of feeding success among depth strata (surface, pycnocline, deep) for larval *Myxodes viridis* at different developmental stages. PIPG = number of prey items per gut, PLPG = average prey length per gut, and TPVG = total prey volume per gut. H = Kruskal-Wallis ANOVA test / Comparación de los índices de éxito alimentario entre estratos de profundidad (superficie, pincloina, profundo) para larvas de *Myxodes viridis* en diferentes estados de desarrollo. PIPG = número de ítems presa por estómago, PLPG = longitud promedio de presa por estómago, y TPVG = volumen total de presas por estómago. H = prueba ANOVA de Kruskal-Wallis**

	Preflexion		Flexion		Postflexion	
	H	P	H	P	H	P
PIPG	0.27	0.874	0.93	0.628	1.95	0.376
PLPG	3.51	0.173	0.86	0.650	3.63	0.163
TPVG	3.92	0.141	0.61	0.736	0.81	0.665



**Figure 6. Feeding success of larval *Myxodes viridis* at different developmental stages, measured as the total number of prey items per gut, total volume of prey items per gut and average prey size per gut. S = surface stratum, P = pycnocline stratum, D = deep stratum. Larvae were collected at the down-estuary station (San Carlos) / Éxito alimentario de larvas de *Myxodes viridis* a diferentes estados de desarrollo, medidas como el número de ítems presa por estómago, volumen total de presas por estómago y tamaño promedio de presas por estómago. S = estrato superficial, P = estrato de la pincloina, D = estrato profundo. Las larvas fueron recolectadas en la estación estuario-abajo (San Carlos)**

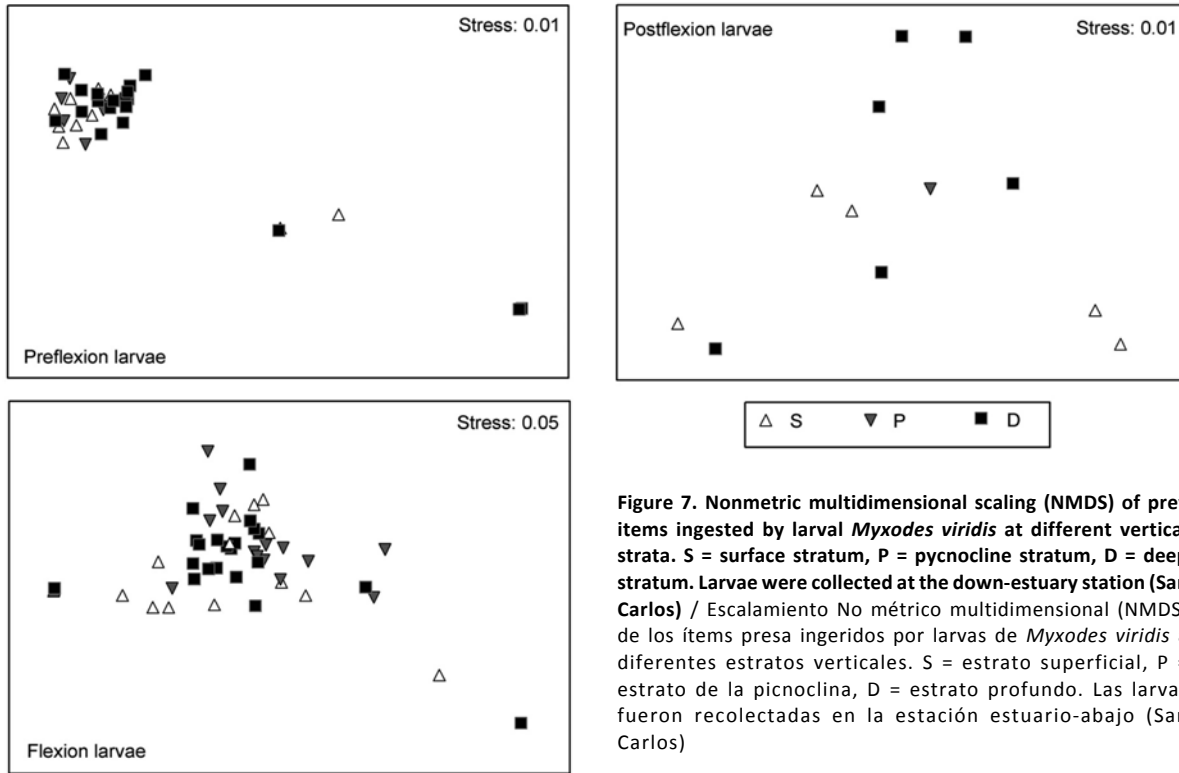


Figure 7. Nonmetric multidimensional scaling (NMDS) of prey items ingested by larval *Myxodes viridis* at different vertical strata. S = surface stratum, P = pycnocline stratum, D = deep stratum. Larvae were collected at the down-estuary station (San Carlos) / Escalamiento No métrico multidimensional (NMDS) de los ítems presa ingeridos por larvas de *Myxodes viridis* a diferentes estratos verticales. S = estrato superficial, P = estrato de la pycnoclina, D = estrato profundo. Las larvas fueron recolectadas en la estación estuario-abajo (San Carlos)

larval *M. viridis* at the flexion stage, differences in diet were also detected (global  $R = 0.111$ ,  $P = 0.001$ ). Similar to the preflexion larvae, differences in diet were observed between flexion larvae from surface and deep strata ( $R = 0.135$ ,  $P = 0.002$ ) and between larvae from the pycnocline and deep strata ( $R = 0.138$ ,  $P = 0.001$ ). Dissimilarities were caused by nauplii (25.94%), copepodites (22.79%) and copepod eggs (11.10%) in the case of larvae from the surface and deep strata, and by nauplii (25.86%), copepodites (21.73%) and copepodite remains (9.97%) for larvae collected in the pycnocline and deep strata. In the case of postflexion larvae, no significant differences were detected in diet among strata (global  $R = -0.120$ ,  $P = 0.667$ ).

## DISCUSSION

The early life stages of *Myxodes viridis* (Clinidae) collected from the Corral Bay estuary during the austral spring 2009 were more abundant at the down-estuary station during the ebb phase of the tidal cycle. Larvae change from omnivory to carnivory as they grow, selecting larger prey (from dinoflagellates to large copepods) as the mandible length increases. Foraging

occurred mostly during the ebb phase of the tidal cycle. Although the feeding success was similar for each developmental stage at all depths, the data suggest that predation in the first 3-4 m (surface and pycnocline) was on different prey items than in deeper waters, at least for the preflexion and flexion stages. Therefore, the pycnocline may affect the composition of the diet of small, larval *M. viridis* but not the size or number of prey ingested by larvae in the estuary.

Most larval *M. viridis* were captured at San Carlos (down-estuary) during the ebb phase. The greater larval fish abundance associated with ebbing conditions may be a tactic of the species to increase advection down the estuary and to the nearby shelf waters. According to Whitfield (1989), pulsed hatching on the ebb tide would enhance the movement of larvae into the ocean for the marine phase that is required by some species for further development. Some coastal fish larvae from Australian (*Favonigobius lateralis*, *Pseudogobius olorum*, Neira & Potter 1992) and South African estuaries (*Omobranchus woodi*, *Psammogobius knysnaensis* and *Syngnathus acus*, Strydom & Wooldridge 2005) are also flushed out of the estuaries on ebb tides. Some species return to the estuary by sinking deep in the water column during the later stages

of larval development, whereas others maintain a surface position and exploit gyres or wind-driven countercurrents for retention on the inner shelf near the parent estuary (Epifanio 1988).

The increase in feeding during ebbing conditions has been observed in labrisomid species from the Galapagos, *Dialommus fuscus* (Nieder 2001). This species forages in the wet sections of the intertidal habitat during low tide. Other blennies show an opposite trend, with greater feeding activity during the rising tide than during the ebbing tide (Faria & Almada 2008). Other species, such as the juvenile *Dicentrarchus labrax*, colonize intertidal marsh creeks during the flood phase, forage on resident amphipods, and return to coastal waters during the ebb phase (Laffaille *et al.* 2001). The asymmetry in food ingestion may reflect a trade-off between maximization of feeding time and the ability to reach the low-tide shelters in time, to avoid the risk of stranding in unsuitable environments.

The change from omnivory to carnivory has been observed in fish larvae from several coastal environments (cod *Gadus morhua*, Kane 1984; sand lance *Ammodytes americanus*, Monteleone & Peterson 1986; anchovy *Engraulis ringens*, sardine *Strangomera bentincki*, Llanos-Rivera *et al.* 2004), and it has been considered an opportunistic behavior. While some species show omnivory throughout the larval stage in estuarine systems, some other fish larvae are exclusively carnivorous (herring *Clupea harengus*, Fox *et al.* 1999; lightfish *Maurollicus parvipinnis*, Landaeta *et al.* 2011). Juveniles and adults of *Myxodes viridis* are carnivorous, preying mostly on crabs, amphipods and harpacticoid copepods (Stepien 1990, Muñoz & Ojeda 1997). Additionally, differences in the composition of prey and the prey size among developmental stages may indicate that intraspecific competition is reduced or absent. Generally, small larvae are poor predators relative to the larger larvae due to their poorer vision, cognitive capacity and swimming activity; thus, they are more vulnerable to variations in food availability (Sassa & Tsukamoto 2012).

At each developmental stage, larvae were similarly successful at ingesting their prey at different depths of the estuary. Although several studies have highlighted the role of clines in the aggregation of zooplankters and their food, creating biological hotspots (Raby *et al.* 1994, Grønkjær & Wieland 1997, Woodson & McManus 2007, Tapia *et al.* 2010, Woodson *et al.* 2012), a strong halocline (*i.e.*, pycnocline) occurred throughout the study at both stations, and this oceanographic feature did not affect the feeding success of fish larvae. Pycnoclines may

provide optimal conditions for phytoplankton growth and can be regions of enhanced phytoplankton biomass as a result of both passive and active organism responses (McManus & Woodson 2012). Differences in the prey composition of *M. viridis* larvae were identified by the multivariate analyses; these analyses suggested different prey fields in the surface/halocline and deep strata of the estuary that can be utilized by fish larvae in a successful way, irrespective of the physical conditions of the water column (*i.e.*, heavy turbulence in the surface and deep strata and greater stability in the pycnocline).

The study area, the Corral Bay estuary, is utilized as spawning and early nursery grounds by a series of marine and estuarine fishes (sardine *Strangomera bentincki*, silverside *Odontesthes regia*, Vargas *et al.* 2003) and crustaceans (crabs *Cancer edwardsii* and *C. coronatus*, Pardo *et al.* 2010, 2011, 2012). In this estuary, crab settlement is related to the advection of warm waters toward the coast and/or coastal upwelling, but it is species-specific (Pardo *et al.* 2012); microtides may affect larval fish composition, and in our case, the feeding incidence of fish larvae. The absence and/or reduced abundance of larval *M. viridis* in the up-estuary station may be regulated by or associated with salinity tolerance.

Studies of larval ecology in estuaries from the southeastern Pacific Ocean are scarce, and this is the first investigation to focus on the trophic preferences of a larval clinid in a microtidal estuary. It is important to continue investigating the role of estuaries as nursery areas for marine, non-resident fish species, especially in terms of growth and survival.

In conclusion, larvae of the clinid *Myxodes viridis* did not display estuarine dependence in their diet in Corral Bay estuary. They were collected during the austral spring, mainly during the ebb phase of the tidal cycle, a period when they increase their capability to ingest prey items. During the preflexion and flexion stages, *i.e.*, when swimming capabilities are reduced, they predate on small phyto- and zooplankton, and as the larvae grow, they become strictly carnivorous.

During the study period, the microtidal estuary displayed a prominent halocline at 2-3 m depth; however, this oceanographic feature did not affect the feeding success of larval *M. viridis*. If the pycnocline concentrates food items, fish larvae were unable to increase their prey ingestion; alternatively, a similar feeding success may indicate that the larval clinids forage equally in the mixed and deep layers as well as the pycnocline.

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