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Threespine stickleback (*Gasterosteus aculeatus*) diet in El Rosario River estuary, its southernmost North American locality

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SUMMARY

The diet composition of the threespine stickleback (*Gasterosteus aculeatus*) at the limit of its southernmost distribution in North America (El Rosario River estuary, Baja California, Mexico) was analysed throughout five sampling events covering the wet season (November 2008, 2009 and 2011) and dry season (May 2010 and June 2013). The analysis of stomach contents of 159 threespine sticklebacks revealed 14 prey categories, of which chironomid larvae dominated the diet in most of sampling events (55-89%) except for June 2013, where leptophlebiid mayfly nymphs (73%) were mainly consumed. Differences in diet composition among size-classes and between sexes were dependent on the season (wet or dry), showing no relation with the prey size consumed. This study corroborates the high consumption of chironomid larvae as reported for the most part of its Holarctic distribution.

Keywords: *Gasterosteus aculeatus*, diet composition, distribution limits, Baja California

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INTRODUCTION

The threespine stickleback (*Gasterosteus aculeatus* Linnaeus, 1758, henceforth simply stickleback) is distributed across the boreal and temperate regions of the northern hemisphere, inhabiting coastal marine waters, estuarine waters and an ample variety of freshwater habitats (Bell & Foster, 1994). In North America, this fish ranges from the Bering Strait, Alaska (Eigenmann, 1892), to El Rosario River, Baja California, Mexico (Ruiz-Campos et al., 2014; Fig. 1). The stickleback has declined in both abundance and distribution range in northwestern Baja California due to the progressive anthropogenic alteration of its habitats (Ruiz-Campos et al., 2014; Ruiz-Campos & Varela-Romero, 2016). Although the stickleback is not threatened at the global scale according to the criteria of the IUCN (https://www.iucnredlist.org/species/8951/76576912), the species in considered to be endangered in Mexico (SEMARNAT (2010).

Studies on the diet of the stickleback in different regions of its Holarctic distribution indicate that it feeds mainly on aquatic invertebrates, especially chironomid larvae, copepods, cladocerans and ostracods (e.g. Wooton, 1976; Sandlund et al., 1992; Sánchez-Gonzáles et al., 2001; Niksirat et al., 2010; Sánchez-Hernández et al., 2012; Richardson et al., 2017; Roch et al., 2018). The feeding ecology of this species is well documented in many localities in both North America and Eurasia, including a locality in northern Mexico (El Descanso lagoon) just 44 km south of the US-Mexico border (Sánchez-Gonzáles et al., 2001), where the diet composition changes seasonally with sex and size classes in relation with prey availability. However, no information is available on the feeding ecology of this species in its southernmost distribution limit in North America. The stickleback faces severe conservation problems in this area, due to habitat reduction generated by overexploitation of aquifers in the lower basin via agricultural activities (Ruiz-Campos et al., 2014). Here, we describe the diet composition of the stickleback in the El Rosario River estuary, Baja California, and compare it among sexes, size classes and seasons (wet and dry).

Figure 1. Threespine Stickleback (Gasterosteus aculeatus) from estuary of El Rosario River, Baja California, Mexico. Female (A) and male (B) in breeding coloration.

STUDY AREA

The present research was conducted in El Rosario River estuary, at the southern end of the Mediterranean region of Baja California (Fig. 2). This estuary is a 1 km long, 200 m wide and 2.3 m deep lagoon, with its mouth blocked by a sandbar (Ruiz-Campos & Rodríguez-Meraz, 1993). During the high winter tides, seawater penetrates the lagoon, generating a salinity gradient within the system (Ruiz-Campos et al., 2000). This region has a Mediterranean-type climate, with a rainy and cold period during autumn and winter (Fig. 3A), and a hot and dry one during spring and summer (Fig. 3B). The annual mean temperature and precipitation of this region oscillate from 16-18 °C and 300-375 mm, respectively (Delgadillo, 1998; Vanderplank, 2011).

The river bottom is sandy-muddy, and the vegetation on the banks is composed of halophytes, mainly *Salicornia bigelovii* and *Juncus acutus* with emergent macrophytes upriver, such as *Typha domingensis* and *Scirpus californicus* (Ruiz-Campos et al., 2005). The arboreal vegetation is dominated by the exotic, *Tamarix ramossisima* (Ruiz-Campos et al., 2000), while the fish community features the stickleback, the exotic *Gambusia affinis* (Baird & Girard, 1853), *Mugil cephalus* Linnaeus, 1758, *Atherinops affinis* (Ayres, 1860), *Hyperprosopon argenteum* (Gibbons, 1854) and *Amphistichus argenteus* Agassiz, 1854 (Ruiz-Campos et al., 2000; Ruiz-Campos et al., 2014).

Water temperature (°C), salinity (‰), dissolved oxygen (mg/l) and pH were recorded at each site of placing of traps using a Hydrolab Scout 2 multi-analyzer (Hydrolab Co., Austin, Texas). The lowest values of temperature (14.5 °C in November 2009), salinity (2.2 ‰ in November 2011) and pH (8.8 in November 2008) were recorded during the wet season (Fig. 4). The dry season was associated to the highest salinity (6.2 ‰) and temperature (22.7 °C) values, registered in May 2010, along with the highest pH (10.4) in June 2013. The dissolved oxygen values were highest in May 2010 (11 mg/l), but had decreased significantly by the next sampling visit (1.6 mg/l in November 2011).

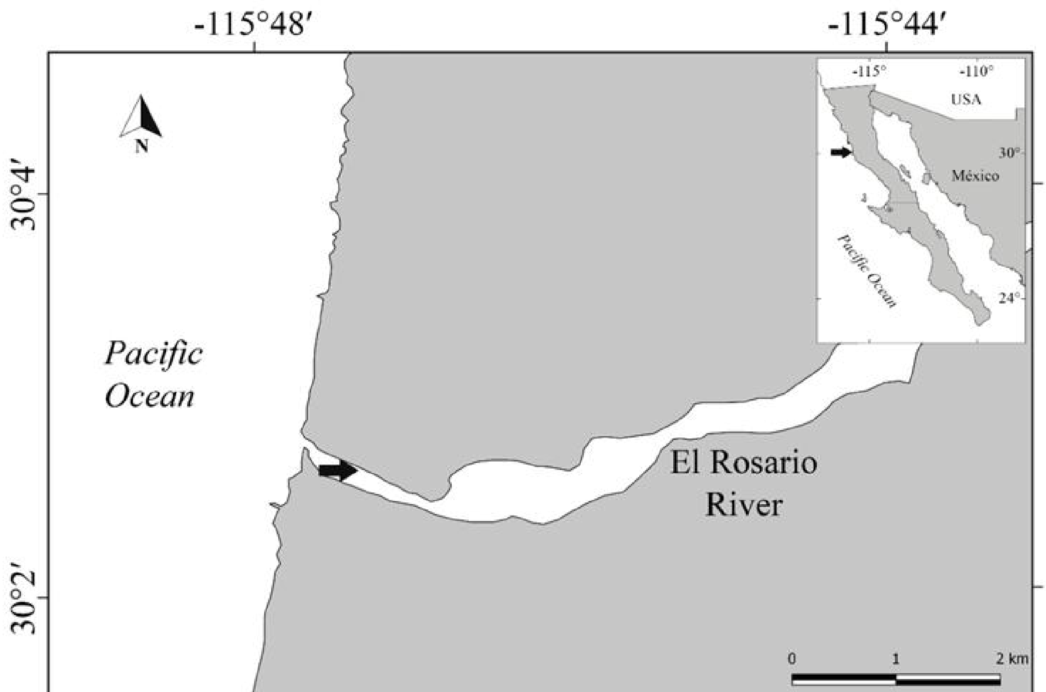

Figure 2. Map and location of the study area at the estuary of El Rosario River, Baja California, Mexico.

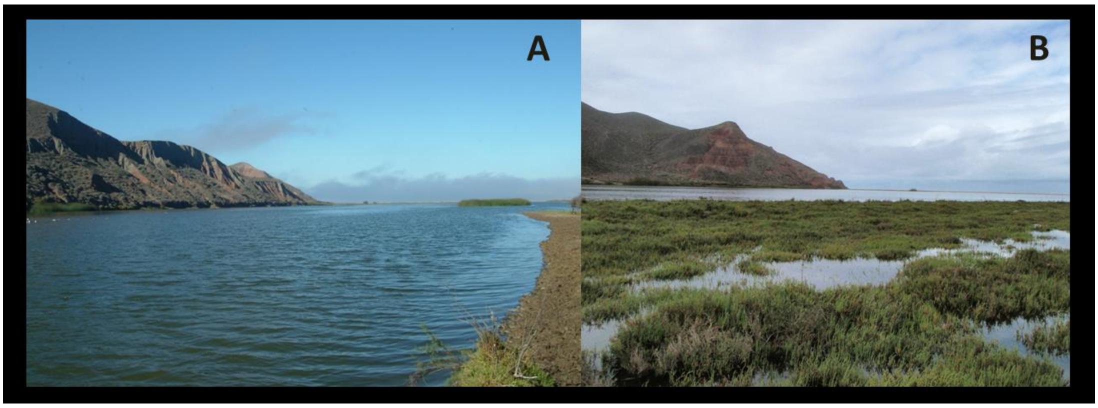

Figure 3. General aspect of the estuary of El Rosario River in Baja California, Mexico, during the dry season (A) and the wet season (B).

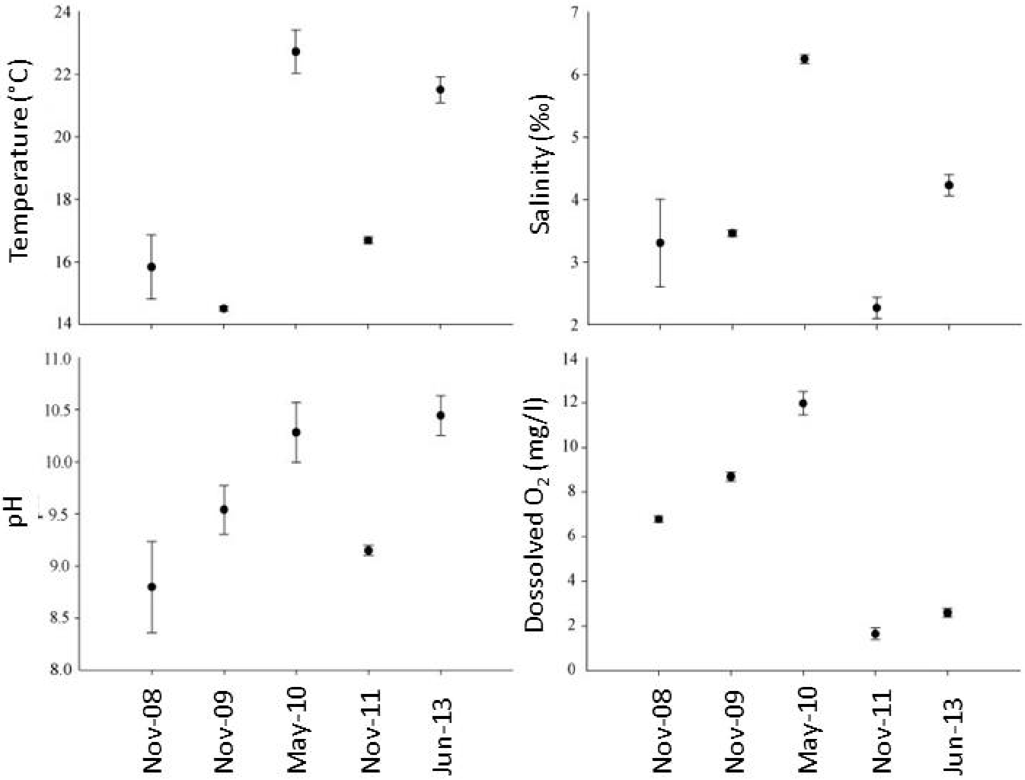

Figure 4. Average values of physical-chemical parameters registered at the estuary of El Rosario River, Baja California, during five sampling events, from November 2008 to June 2013. November sampling events correspond to the wet season and those in May and June are from the dry season.

METHODS

Five sampling events were conducted at El Rosario River estuary, three of them during the wet season (November 2008, November 2009 and November 2011) and two during the dry season (May 2010 and June 2013). Fish were captured using ten minnow traps (45 cm long × 23 cm in diameter, 6.4 mm mesh size, and a funnel at both ends with a 2.2 cm diameter opening). The traps were placed at a 500 m river stretch where previous samplings had recorded the presence of stickleback (Ruiz-Campos et al., 2000). Traps were set at intervals of 50 m, using marshmallows as visual attractors, as described by Ruiz-Campos et al. (2006, 2014). The average deployment time for traps was 15 h.

Although several methods of removing the stomach contents from live fish have been used to study the diet of endangered species (e.g., stomach flushing techniques, emetics, etc.; see Sánchez-Hernández et al., 2010), these procedures are difficult to apply in small fish (Hyslop, 1980; Sánchez-Gonzáles et al., 2001) as the stickleback. Therefore, fish were sacrificed immediately after capture and stored first in formalin (40%) for several minutes to detain digestion, followed by 10% buffered formalin. Regurgitation was not observed. After at least seven days, stickleback individuals were transferred to 50% isopropyl alcohol until examination of stomach content in the laboratory.

The number of stomachs analysed per sampling event was determined by means of a cumulative prey diversity curve (Ferry & Cailliet, 1996), being this number from 27 to 35. These samples sizes were assumed to be sufficient to provide representative information on the diet composition of stickleback for each sampling event.

In the laboratory, we measured the standard length (SL) of each and established two size classes, based on body sizes frequency distribution (C1= 29-36 mm SL and C2= 37-46 mm SL). The sex of the fish was determined by macroscopic inspection of the gonads. We measured the mouth width of each fish with a 0.01 mm precision.

The content of each stomach was washed in a Petri dish, in which the different prey categories were separated, identified and counted. The prey count considered each head as an individual. The volumetric contribution of each prey type was further determined by calculating the mean dimensions according to their closest geometric shape (Hyslop, 1980).

The index of relative importance (IRI; Pinkas et al., 1971) was used to describe the diet composition of the stickleback, because, according to Hyslop (1980), the diet is best represented when both the amount (number) and volume of prey items are considered in the analysis. The IRI was calculated as:

$$IRI = (\%N + \%V) \times \%FO$$

, where %N is the percentage numerical abundance, %V is the percentage volume and %FO is the percentage frequency of occurrence. For comparative purposes, IRI was standardized as %IRI (Cortés, 1997) using the equation:

$$\%IRI_i = (IRI_i / \sum IRI_i) \times 100.$$

The similarity of diet composition among sampling events, size classes and sexes was estimated using Schoener's overlap index (1970) as:

$$\alpha = [1 - 0.5 (\sum | P_{xi} - P_{yi} |)] \times 100$$

, where Pxi is the proportion of the prey type i in the diet of group x (e.g. sampling event x) and Pyi is proportion of the prey type i in the diet of group y (e.g. sampling event y). This index is appropriate in absence of data on prey availability (Wallace, 1981). Diet overlap was considered significant for values of $\alpha \geq 60\%$ (Zaret & Rand, 1971). In order to illustrate the similarity of diet composition among sampling events, we built tree diagrams based on Chevichev's distance metric (dissimilarity) using Statistica 7.0 software (StatSoft, Inc., Tulsa, OK, 2002).

Finally, a Spearman's nonparametric correlation (Siegel & Castellan, 1995) was used to determine if the average size of prey consumed by individual fish is related to its mouth size, under the assumption that the higher energetic requirements of larger individuals would involve a higher consumption of large prey (Townsend and Winfield, 1987).

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RESULTS

Diet composition

The stomach content analysis conducted on 159 sticklebacks, ranging from 29 to 46 mm SL, and allowed the identification of 14 major prey categories (Table 1). Chironomid larvae dominated the diet in terms of %IRI (84.7%), with very little contribution from the other prey categories (> 4% each). During the wet season, the contribution of chironomid larvae oscillated between 54.7% (November 2011) and 94.7% (November 2009), while in the dry season chironomid larvae (73%) and corixids (24.9%) dominated the diet in May 2010 and leptophlebiid mayfly nymphs (71.4%) dominated the diet in June 2013 (Fig. 5).

Both male and female stickleback fed mainly on chironomid larvae (78% and 89%, respectively, Table 1). The relevance of chironomid larvae in the diet of female individuals decreased over time, dropping from 98% in November 2008 to 43% in November 2011. In June 2013 mayfly nymphs were the main prey (42%) of female stickleback. Males, on the other hand, preyed mainly on chironomid larvae in November 2009 (91%), May 2010 (73%) and November 2011 (65%), although this prey category was less important in November 2008 (46%), when males consumed a lot of fish eggs (42%), and was entirely absent in June 2013, when the diet was dominated by mayfly nymphs (85%).

The diet composition of size-class C1 (29-36 mm SL) was dominated by chironomid larvae from November 2008 (84%) to November 2011 (72%), with a peak in dominance in November 2009 (93%). However, in June 2013, chironomid larvae represented only 8% of the diet of small stickleback individuals, while ostracods and mayfly nymphs (Leptophlebiidae) made up 28% and 51% of the diet, respectively. The diet of size-class C2 (37-46 mm SL) also exhibited dominance by chironomid larvae in November 2008 (90%), November 2009 (95%) and May 2010 (70%). However, in November 2011, size-class C2 preyed less on chironomid larvae (12%) and more on copepods (37%) and cladocerans (20%). In June 2013, diet composition was strongly dominated by mayfly nymphs (93%), while chironomid larvae and ostracods represented only 3% and 4% of the diet, respectively (Table 1).

Then relationship between the size of fish mouth and the size of consumed prey assessed for all the sampling events combined showed that the average prey volume consumed by the stickleback was independent of fish mouth size (r_s= 0.06, P=0.45). This relationship was equally non-significant for all sampling events, except for a slightly significant positive correlation found in June 2013 (r_s= 0.4, P= 0.038).

Diet overlap

There was a significant dietary overlap (α ≥ 60%) among the majority of sampling events, except for that conducted in the dry season of June 2013 (Table 2), when the diet was notably dissimilar (Fig. 6), arguably due to the high consumption of mayfly nymphs. The diet overlap between the sexes was significant for November 2009 (94.5%) and May 2010 (95.7%), but non-significant diet in November 2008 (48.5%), November 2011 (50.1%) and June 2013 (49.8%) (Table 2). Between size-classes diet was significantly similar in November 2008 (92%), November 2009 (96.7%), and May 2010 (94.7%), but not in November 2011 (27.2%) and June 2013 (58.3%; Table 2).

DISCUSSION

The results of the present study showed that the diet composition of the stickleback at the southernmost limit of its geographical distribution in North America was dominated by chironomid larvae. This high contribution of chironomid larvae has also been reported in other studies conducted on this species throughout its Holarctic distribution (Hynes, 1950; Sánchez-Gonzáles et al., 2001; Niksirat et al., 2010; Richardson et al., 2017; Roch et al., 2018).

Table 1. Index of relative importance (%IIR) of prey taxa consumed by threespine stickleback at the estuary of El Rosario River, Baja California, during five sampling events (November 2008 to June 2013), in terms of overall diet, and diffrerentiated by sexes and size-classes (C1= 29-36 mm SL, C2= 37-46 mm SL). Numbers in bold indicate the most important prey taxa per category, N= counted prey and P= mean prey size (mm^3). Prey categories acronyms: CLA- Cladocera; OST- Ostracoda; COP- Copepoda; CHI-L- Chironomidae larvae; CHI-P- Chironomidae pupae; LEP- Leptophlebiidae; COR- Corixidae; Misc- Miscellaneous (including Decapoda, P=6.18, Baetidae, P=0.14, Sialidae, P=1.25, Coenagrionidae, P=3.47, Oribatida, P=0.02, and Ephydridae, P=4.26). The number of identify prey taxa (N taxa) and the number of stomachs analysed (N Stom) are also presented. Asterisks (*) denote %IRI< 1%.

		CLA	OST	COP	CHI-L	CHI-P	LEP	COR	Fish eggs	Misc	N Taxa	N Stom
TOTAL	%IRI	0.3	3.5	1.7	**84.7**	1.3	3.7	3.1	1.4	0.2	9	159
	N	86	243	337	1,257	47	46	310	201	47		
	P	*	0.13	0.03	1.13	3.45	2.74	0.10	0.85	M		
Females	nov-08		*	1	**98**	*		*	*	*	7	19
	nov-09	*	3		**96**				*	*	5	18
	may-10		*		**70**	*		26	*	*	6	7
	nov-11	14	8	15	**43**	2	9		3	6	11	24
	jun-13	3	29		25	*	**42**			*	6	15
	Total	*	5	1	**89**	*	3	*	*	*	13	83
Males	nov-08		*	5	**46**	*	4		**42**	*	8	12
	nov-09		6	*	**91**	2	*		*	*	7	17
	may-10		*	*	**73**	1		24	*	*	6	25
	nov-11				**65**	**27**	5			3	5	10
	jun-13	2	5	8			**85**	*		*	6	12
	Total	*	2	2	**78**	3	5	7	2	*	12	76
Size-Class C1	nov-08		2	3	**84**	2	2		5	2	8	8
	nov-09		5	*	**93**	2	*				6	14
	may-10			*	**75**	1		23		*	6	19
	nov-11	3	9	*	**72**	5	8			2	9	20
	jun-13	4	**28**	7	8	*	**51**	*		2	9	18
	Total	*	7	2	**80**	2	4	4	*	*	12	79
Size-Class C2	nov-08			2	**90**	*	*	*	8	*	7	23
	nov-09	*	4		**95**	*	*		*	*	7	21
	may-10		*	*	**70**	1		**26**	3	*	7	13
	nov-11	**20**	*	**37**	12	7	6		9	9	12	14
	jun-13	*	4		3		**93**			*	4	9
	Total	*	1	1	**86**	*	4	3	4	*	13	80

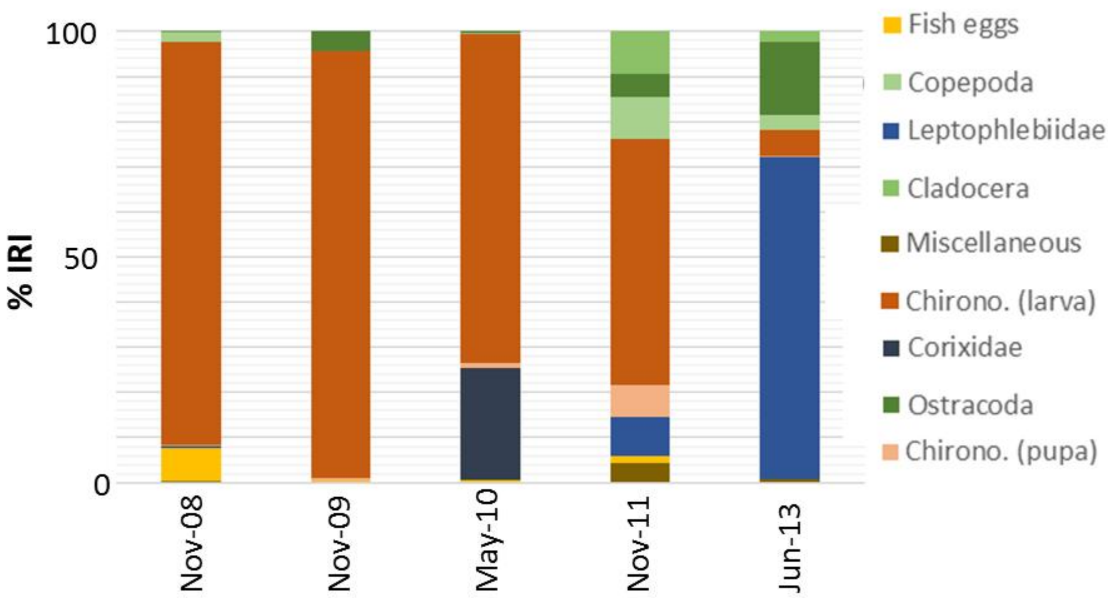

Figure 5. Diet composition (%IRI) of the threespine stickleback (both sexes and size classes combined) during five sampling events between November 2008 and June 2013), at the estuary of El Rosario River, Baja California. November sampling events correspond to the wet season and those in May and June are from the dry season.

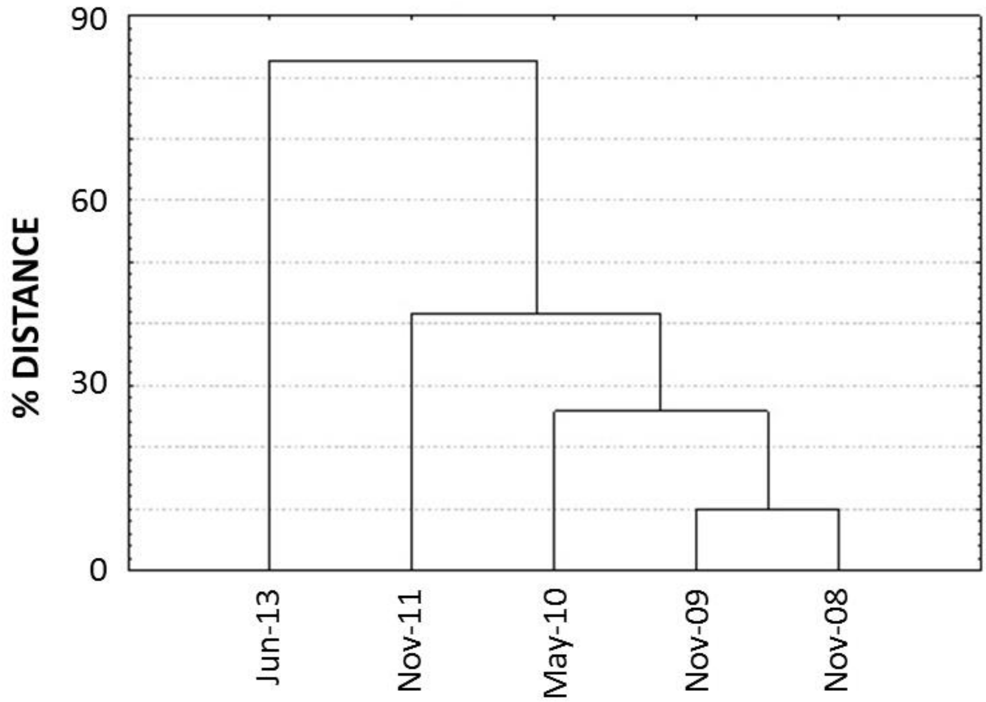

Figure 6. Tree diagram of diet dissimilarity (% distance) of the threespine stickleback (*Gasterosteus aculeatus*) among sampling events in the estuary of El Rosario River, Baja California.

Table 2. Diet similarity (Schoener's overlap index) of threespine stickleback among five sampling events (November 2008 to June 2013), between sexes and between size-classes (C1= 29-36 mm SL, C2= 37-46 mm SL) at the estuary of El Rosario River, Baja California. Numbers in bold indicate significant overlap values (α = >60%, Zaret and Rand 1971).

	Nov-08	Nov-09	May-10	Nov-11	Jun-13	Overall
Nov-08		**90.0**	**73.9**	59.6	8.8	
Nov-09			**74.3**	**60.0**	10.5	
May-10				56.8	6.5	
Nov-11					26.4	
Sexes	48.5	**94.5**	**95.7**	50.1	49.8	**86.3**
Size-Classes	**92.0**	**96.7**	**94.7**	27.2	58.3	**90.1**

Previous research had also shown seasonal variation in the diet of the stickleback, with higher zooplankton consumption during autumn and winter, while the chironomid larvae and other benthic prey dominated the diet during spring and summer (Thorman & Wiederholm, 1983; Allen & Wootton, 1984; Snyder, 1984; Sánchez-Gonzáles et al., 2001; Niksirat et al., 2010). In our study, the diet of the stickleback during the sampling events conducted in the autumn (wet season) was dominated by chironomid larvae. In this same wet season, particularly in November 2011, a higher number of zooplankton prey categories (cladocerans, copepods and ostacods) was observed, coinciding with the lowest average salinity value (2.2‰).

Our study found that stickleback diet was very similar among most of samplings events due to the homogeneity in the types and proportions of consumed prey, particularly of chironomid larvae (54.7% to 94.7%). However, for June 2013 (dry season) the diet was very different due to a significant consumption of leptophlebiid mayfly nymphs (71.4%) and ostracods (16.2%).

The presence of corixids in the diet of stickleback in May 2010 (dry season) was probably favoured by the scarcity of main prey tyoes such as chironomids and mayflies due to changes in salinity (Kefford et al., 2003; Ahmadi et al., 2011; Zinchenko & Golovatyuk, 2013). Corixids are very common organisms in estuarine environments due to their high tolerance to salinity changes (Knowles & Williams, 1973; Piscart et al., 2005).

In November 2011, there was a peak in zooplankton consumption and a decrease in the importance of chironomid larvae in the diet, which seems to be associated to an increase in the consumption of leptophlebiid mayfly nymphs. This pattern became more accentuated in June 2013, when leptophlebiid mayfly nymphs dominated the diet while chironomid larvae were consumed at minimal levels. While the mayflies have been rarely reported in the diet of the stickleback, Allen and Wootton (1984) reported them as important prey items in the summer diet of this species in a lake in Wales (UK). As the highest densities of mayfly nymphs have also been reported during spring and autumn in the mountain streams of the Mediterranean region of Baja California (Ruiz-Campos, 2017), their presence in the diet of the stickleback could indicate an opportunistic feeding strategy promoted by an increase in the availability of this prey. The change in the composition of stickleback diet at our study site confirms the plasticity of this anadromous species in exploiting dietary resources (Snyder, 1984; Stephens & Krebs, 1986; Sánchez-Gonzáles et al., 2001; Araújo et al., 2008).

Diet similarity between sexes was high during the sampling events of November 2009 and May 2010, in agreement with previous sutdies (cf. Worgan & FitzGerald, 1981; Snyder, 1984; Sánchez-Gonzáles et al., 2001). However, in the other three sampling events, diet composition was significantly different between sexes. In November 2008, these differences in the diet were mainly due to males preying heavily upon fish eggs (42%). Egg cannibalism has been reported for this species during the spawning season (Allen & Wootton, 1984; Snyder, 1984; Sánchez-Gonzáles et al., 2001; Richardson et al. 2017) and more often in males (Worgan & FitzGerald, 1981; Hyatt & Ringler, 1989). It is therefore possible that a high consumption of fish eggs by male sticklebacks in our study area could be indicative of cannibalism (Hyatt & Ringler, 1989).

In our study, a higher number of prey categories was found in females in November 2011, while males seemed to focus on one or two preferred prey categories in November 2011 (chironomid larvae and pupae with 65% and 27%, respectively) and June 2013 (mayfly nymphs with 85%). Differences in trophic niche breadth between sexes of this species have been reported in previous studies (Worgan & FitzGerald, 1981; Sánchez-Gonzáles et al., 2001) and could be the result of low prey availability, which generates intraspecific competition for food and, consequently, dietary variation between groups with different phenotypic and behavioural characteristics (Svanbäck & Bolnick, 2007; Araújo et al., 2008).

Trophic similarity between size classes was significant during the first three sampling events. In November 2011, however, small fish fed mainly on benthic prey (mayfly nymphs – 72%) while large ones consumed a high percentage of zooplankton (cladocerans 20% and copepods 37%), which could indicate a segregation by size in the water column. In June 2013, even though both size classes fed mainly on benthic prey, the diet of small individuals included a wide amount of small prey items (ostracods, 28%), while large fish focused on large prey (mayfly nymphs, 71%), thus maximizing energetic intake (Townsend & Winfield, 1985). As a result, June 2013 was the only sampling event exhibiting a significant relationship between the average volume of prey consumed by the stickleback and mouth size.

In summary, this study corroborates the dominance of chironomid larvae in the diet of the stickleback at the southernmost limit of its Holarctic distribution. Our findings show that diet composition of the stickleback varies seasonally and in relation to sex and size.

AUTHOR CONTRIBUTIONS

Both authors lead the writing and analytical procedures. GRC lead the field data collection

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