



## Assessment of an ichthyoplankton net operated at different velocities and durations in the Paraná River

By M. I. Gómez<sup>1</sup>, J. A. Calcagno<sup>1,2</sup> and C. M. Fuentes<sup>3</sup>

<sup>1</sup>*Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina;* <sup>2</sup>*Consenjo Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina;* <sup>3</sup>*Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Mar del Plata, Argentina*

### Summary

The aim of this work was to evaluate the performance of a conical ichthyoplankton net under the most common range of sampling conditions found in the Paraná River. Three sampling velocities in the range of 0.3–1.4 m seg<sup>-1</sup> and four sample durations (2, 4, 8 and 16 min) were simulated from two different experiments carried out in surface and bottom waters of the main channel during daylight hours. Neither significant differences nor trends were found in larval fish densities in various stages of development under the conditions studied. Results are discussed in the context of the natural ichthyoplankton abundance variability. The results support the hypothesis that in turbid waters larvae are not visually able to detect the net and that in the size range sampled, they either cannot mechanically detect the gear or have not developed enough swimming ability to efficiently evade it. The results of this work may contribute to the interpretation of the abundance data in ichthyoplankton studies in South American rivers with similar ranges of transparency, flow and larvae development.

### Introduction

Analyses on composition and abundance of ichthyoplankton are subject to errors derived from sampling procedures. Net avoidance and extrusion are important factors accounting for biases in abundance estimates of the larvae (Clutter and Anraku, 1968; Thayer et al., 1983; Urho, 1997).

The stimuli that trigger net avoidance by planktonic organisms can be both visually and mechanically driven (Fleminger and Clutter, 1965; Fuiman and Poling, 1997). The pressure waves on the mouth of the net (potentially enhanced by both clogging and sampling velocity) may significantly increase perception of the net by larvae (Fleminger and Clutter, 1965; Thayer et al., 1983; Fuiman and Poling, 1997). Additionally, water flow in passive sampling or tow speed in active sampling (Vannucci, 1968; Schnack, 1974; Thayer et al., 1983) as well as sample duration (Schnack, 1974) increase the pressure through the mesh (Clarke, 1983, 1991), and can produce extrusion of smaller larvae.

As in other studies in which the advantage of the typical unidirectional flow is taken, passive sampling is a common practice in ichthyoplankton investigations of the Paraná River (Oldani, 1990; Fuentes and Espinach Ros, 1998; Rossi, 2001; Fuentes et al., 2008). However, in adopting this sampling strategy, the net operator loses control of the sampling speed. Consequently, the velocity at which the larvae address the net

is determined by the river flow. Additionally, sampling time and water transparency have been hypothesized to influence rates of captures in rivers (Gadomski and Barfoot, 1998; Araujo-Lima et al., 2001).

Similar to that found for other turbid rivers (Pavlov et al., 1995; de Graaf et al., 1999; Araujo-Lima et al., 2001), no diel patterns of downstream migration of fish larvae have been found in the Middle and Lower Paraná River (Fuentes and Espinach Ros, 1998; Rossi, 2001). For convenience, day sampling is thus normally chosen (Fuentes et al., 1998; Araujo-Lima et al., 2001).

Net evasion has been postulated to be responsible for the fact that captures obtained during daylight are lower than those obtained at night in the Rio Negro River, a high-transparency tributary of the more turbid Amazon River, where these differences have not been found (Araujo-Lima et al., 2001). It is conceivable that the high water turbidity in the middle and lower sections of the Paraná River may 'block' visual stimulus of larvae, and therefore contribute to impair net detection during daylight sampling.

Yet, a considerable degree of variability in water current and organic matter load is spatially and temporarily observed both between and within some sections of the Paraná and other rivers of the basin where abundance of the early stages of valued species is annually quantified (Fuentes et al., 2008). In practice, net clogging in long-duration samples and differences in water flow through a net deployed against the river current could also influence the net performance. In these cases, net avoidance would be triggered by an increased perception of the frontal wave by the larvae as a consequence of a mechanical stimulus. Additionally, the above-mentioned differences in river flow and the long sampling durations (in order to increase the sampled water volume) sometimes chosen could account for the extrusion of less developed larvae.

In order to assess the degree of bias associated with the sampling procedure, the performance of an ichthyoplankton net at different sampling velocities (i.e. larvae approaching velocities) and sample durations (i.e. clogging levels) were evaluated in the Paraná River.

### Materials and methods

#### Study site

Sampling was conducted 300–500 m offshore in the main channel of the Middle Paraná River, off the city of Paraná, Entre Ríos Province, Argentina. At the study site (ca. 31.7°S, 60.5°W), channel width and depth were 1200 and 10–15 m,

respectively. Water transparency (Secchi disc = 20 cm), water temperature (27°C) and caudal (13 000 m<sup>3</sup> s<sup>-1</sup>) were in the range of values commonly found in the middle section of the Paraná River (Drago, 1989).

### Field experiments

The performance of a conical ichthyoplankton net (1 m length, 0.35 m mouth diameter, equidistantly bridled, 300 µm mesh, 60% filtering area and a relationship of filtering area to mouth area of 4.89:1) was evaluated under different sampling velocities (Experiment 1, 0.3–1.4 m s<sup>-1</sup>) and sample durations (Experiment 2, 2–16 min) during daylight hours on 5 January (Experiment 1, n = 9; Experiment 2, n = 5) and 1 February (Experiment 1, n = 5; Experiment 2, n = 5) 2004. Treatment levels were recreated to reflect those commonly carried out previously in Middle and Lower Paraná River ichthyoplankton (Oldani, 1990; Fuentes, 1998; Rossi, 2001).

In Experiment 1, a net operator deployed one net either at the surface (0.5–1 m) or bottom (~1 m from the bottom) of the river from a small pneumatic boat displaced by means of a 120 m rope manually operated from a ship anchored in the river (Fig. 1). The bottom stratum was reached by equipping the net with a 10 kg depressor and maintaining the tension and angle of the rope (Fig. 1), which allowed estimating the depth reached with an acceptable degree of precision. The 'low' velocity (0.3–0.6 m s<sup>-1</sup>) was obtained by slowly releasing the rope towards the main current. The 'medium' velocity (0.6–1 m s<sup>-1</sup>) was obtained by keeping the boat (and then the net) motionless in the river flow; the 'high' velocity (1–1.4 m s<sup>-1</sup>) was obtained by actively recovering the rope against the flow. This way, samples were taken without any disturbance by engine boats or propellers. Each round of samples consisted of two subsequent blocks (surface and bottom) of low, medium and high resulting velocities randomly ordered. Sample duration for all treatments ranged from 2 to 3 min. A total of 14 replicates were taken for each treatment at both depth strata.

In Experiment 2, samples were taken by deploying two paired surface and bottom nets held stationary against water flow (Fig. 1). A total of 10 blocks of replicates were randomly subjected to the treatments (2, 4, 8 and 16 min).

For both experiments, water velocity, water volume and filtering efficiency were estimated by means of an external and an internal flowmeter (General Oceanics, 2030R).

### Sample processing

Samples were fixed in 5% buffered formaldehyde for 48 h, then preserved in 70% ethylic alcohol. The volume of organic matter was determined by decanting all debris from each sample on a 250 cm<sup>3</sup> gauge for 20 min.

Fish larvae were sorted and identified to the lowest taxonomic level possible from each sample, following Nakatani et al. (2001), but only high taxon levels were considered for the analysis. Since visual and mechanoreceptor acuity depends on the developmental stage of the larvae (Leis et al., 1996; Fuiman and Poling, 1997; Stobutzki and Bellwood, 1997), different variables matching those stages were defined for abundance analysis. The variables defined for characin larvae were: newly hatched (NH, SL: 3–5 mm) and un-yolked pre-flexion (PREC, SL: 5–7 mm); those for siluriform larvae were: pre-flexion (PRES, SL: 4–8 mm), no rayed dorsal fin (NRDF, SL: 8–10 mm), rayed dorsal and pectoral fins (RDF, SL: 9–12 mm) and all fins rayed (AFR, SL: 12–17 mm).

### Statistical procedure

As a preliminary test showed no significant effect of the sampling day (Friedman's test,  $P > 0.05$ ), and the variability between blocks of the same day was larger than that for different days, data from each sampling day were pooled for both experiments to ensure the largest sample size for each test.

Differences in larval densities between treatments in both experiments were determined by Friedman's test. Abundances

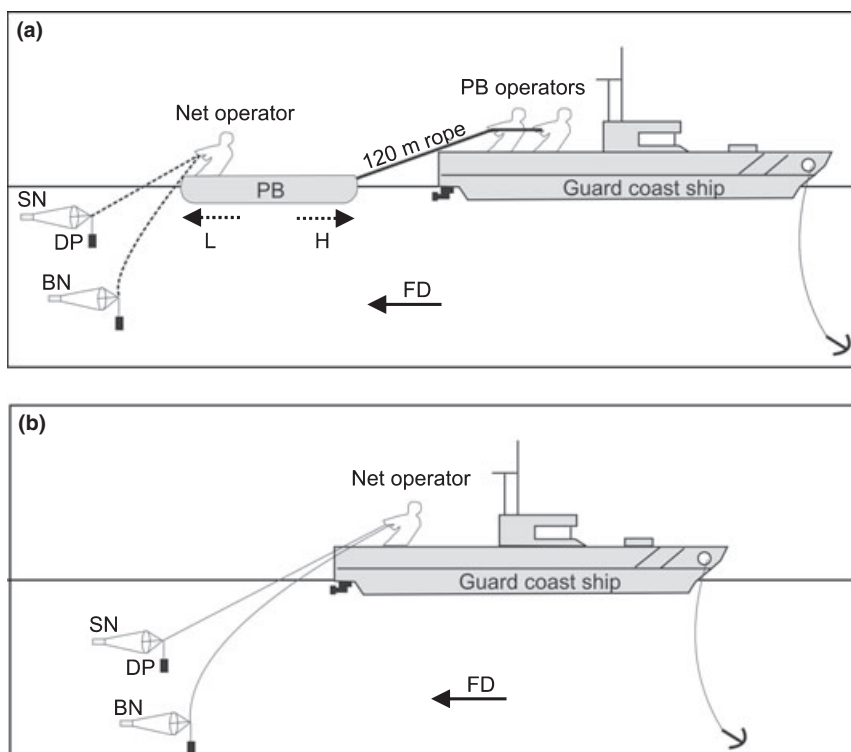


Fig. 1. Experiments 1 (a) and 2 (b) procedures. PB: pneumatic boat; SN: surface net; BN: bottom net; DP: depressor. FD: flow direction; L: low sampling speed; H: high sampling speed. Dashed net ropes: non-simultaneous trials

in all treatments were also averaged within each block and contrasted between depth strata by means of the Mann–Whitney's test (Experiment 1) or the Wilcoxon's test (Experiment 2) in order to assess larval abundance difference between the surface and bottom. Differences in net filtering efficiency, water and organic matter volume between treatments were evaluated by Kruskal–Wallis's test and Dunn's multiple comparisons (Daniel, 1978). Relation between filtering efficiency and organic matter volume was examined by a non-parametric Spearman's correlation for each depth stratum considering all samples.

## Results

### Sampling performance

In Experiment 1, as expected, significantly different sampling velocities were achieved (Table 1). As a secondary effect, filtering efficiency increased with sampling speed (Table 1) showing significant differences between low and high speed at both depth strata (Kruskal–Wallis and Dunn's comparison,  $P < 0.05$ ). However, this increase was not higher than 15% and is attributed to the higher water pressure inside the net in the high-speed trials. Water volume also increased with sampling speed (Table 1), showing significant differences between all treatments at the surface, and between low-speed trials and medium- and high-speed trials at the bottom (Kruskal–Wallis and Dunn's comparison  $P < 0.05$ ).

In Experiment 2, water and organic matter volumes increased significantly with sampling duration, especially in bottom samples (Table 2 and Fig. 2, respectively). Filtering efficiency and organic matter volume showed a significant inverse Spearman's correlation ( $P < 0.05$ ;  $r = -0.76$  for each depth level). As a consequence of clogging, mean filtering efficiency of the net decreased up to 50% of its initial value at 16-min samples (Fig. 2). For both variables at the bottom and

for organic matter volume at the surface significant differences were found only between 2 and 16 min, while filtering efficiency in 16-min samples at surface waters differed from those of 2- and 4-min samples (Fig. 2).

### Larval abundance

None of the experiments or depth strata showed significant differences for any of the abundance variables evaluated ( $P > 0.05$ , Tables 1 and 2). Besides, although a high variability was recorded for all the abundance variables inside each treatment, none of the experiments showed trends for the mean values.

Although larval densities for all groups tended to be lower in the surface than in bottom samples (Fig. 3), the differences were statistically significant only for NH and PREC in Experiment 1, for NRDF and RDF of both experiments, and for NRFF, RDF and ARF in Experiment 2.

### Discussion

Fish eggs and larvae are relatively scarce and aggregated components of planktonic communities, thus ichthyoplankton density is usually highly variable (Hildén and Urho, 1988; Cyr et al., 1992; Pepin and Shears, 1997). Therefore, results of studies on the performance of an ichthyoplankton sampler have to be judged in that context (Hildén and Urho, 1988).

### Net evasion

We found that larval densities were variable for all treatments (Tables 1 and 2). The variability in densities within treatments was partly due to the fact that data sets for each experiment were not entirely obtained from a single date. However, the most important source of variation was attributed to the inherent variation of larval densities in a smaller time scale (between consecutive blocks and within blocks). In a context of high abundance variability, which obscures the differences between treatments, the central tendency of the data (Hildén and Urho, 1988) and size distribution of the larvae (Aron and Collard, 1969) were taken into account in this type of analysis. From this perspective, not only the lack of significant differences, but also the lack of trends in mean densities at different velocities and durations, even for samples taken at the upper-lighted stratum and for the most developed siluriforms (Tables 1 and 2), are coherent with the hypothesis that larvae 'tend not to be able' to visually detect and/or efficiently evade the net in turbid waters during daylight hours. Another conceivable and not excluding hypothesis is that a mechanical stimulus produced by perturbation derived from the net may not have been perceived or efficiently evaded by larvae, which, in the size range sampled, may not have acquired enough mechanoreceptor or swimming capabilities. This hypothesis is supported by the lack of differences in abundance even of the most developed silurids between the 16-min and the 2-min bottom samples (Table 2). After the net accumulated a significant amount of organic matter and markedly reduced its filtering efficiency (Fig. 2), 16-min samples should have produced a higher perturbation at the front of the net, which would have stimulated the larvae to burst away in comparison with that of the lower sampling times (high performance). Such lack of differences could then be attributed to the 'not enough' mechanoreceptor or swimming capabilities of the larvae at their size to evade the net.

Table 1

Mean and standard deviation for sampling velocity (SV), filtering efficiency (FE), water volume (WV) and larval densities (LDEN) for the three ranges of simulated water velocities. First right column: significance level for Friedman's (LDEN) or Kruskal–Wallis's (SV, FE and WV) tests

	Low	Medium	High	P
Surface (n = 14)				
SV (m s <sup>-1</sup> )	0.42 ± 0.15	0.76 ± 0.12	1.16 ± 0.11	0.00
FE	0.68 ± 0.14	0.75 ± 0.09	0.82 ± 0.06	0.00
WV (m <sup>3</sup> )	3.57 ± 1.82	7.47 ± 1.89	10.03 ± 2.11	0.00
LDEN (ind m <sup>-3</sup> )				
NH	1.69 ± 1.25	1.36 ± 1.08	1.60 ± 1.21	0.32
PREC	2.88 ± 1.37	3.08 ± 1.45	3.10 ± 1.03	0.40
PRES	1.85 ± 2.31	1.99 ± 2.06	1.39 ± 0.80	0.61
NRDF	0.14 ± 0.21	0.10 ± 0.14	0.13 ± 0.11	0.50
RDF	0.01 ± 0.04	0.01 ± 0.03	0.01 ± 0.03	0.37
ARF	0.00 ± 0.00	0.02 ± 0.05	0.00 ± 0.00	0.14
TTL	8.12 ± 5.22	8.04 ± 5.94	7.35 ± 3.11	0.93
Bottom (n = 14)				
SV (m s <sup>-1</sup> )	0.34 ± 0.10	0.71 ± 0.12	1.16 ± 0.12	0.00
FE	0.75 ± 0.12	0.78 ± 0.11	0.85 ± 0.05	0.04
WV (m <sup>3</sup> )	3.32 ± 1.33	7.61 ± 1.41	10.19 ± 2.71	0.00
LDEN (ind m <sup>-3</sup> )				
NH	2.76 ± 2.42	3.19 ± 2.62	2.56 ± 2.13	0.63
PREC	4.47 ± 2.23	3.60 ± 1.35	4.04 ± 1.49	0.32
PRES	5.26 ± 6.39	5.49 ± 7.66	6.03 ± 8.01	0.40
NRDF	0.84 ± 0.95	0.52 ± 0.58	0.98 ± 1.49	0.29
RDF	0.28 ± 0.50	0.19 ± 0.34	0.37 ± 0.69	0.23
ARF	0.07 ± 0.16	0.03 ± 0.06	0.05 ± 0.10	0.53
TTL	16.26 ± 10.45	15.78 ± 13.10	16.37 ± 13.00	0.93

Table 2

Mean and standard deviation for water current (WC), water volume (WV) and larval densities (LDEN) for different sample durations (in minutes). First right column: significance level for Friedman's (LDEN) or Kruskal–Wallis's (WC and WV) tests

	2	4	8	16	P
<b>Surface (n = 10)</b>					
WC (m s <sup>-1</sup> )	0.89 ± 0.36	0.82 ± 0.38	0.82 ± 0.33	0.84 ± 0.34	0.71
WV (m <sup>3</sup> )	6.41 ± 2.72	11.38 ± 4.98	21.52 ± 7.46	31.92 ± 12.44	0.00
LDEN (ind m <sup>-3</sup> )					
NH	10.52 ± 15.50	11.86 ± 16.95	11.50 ± 17.1	13.68 ± 20.67	0.06
PREC	10.67 ± 11.98	13.16 ± 17.61	10.71 ± 13.0	13.20 ± 14.64	0.25
PRES	7.24 ± 11.37	7.83 ± 10.65	7.62 ± 10.62	8.96 ± 12.93	0.24
NRDF	0.34 ± 0.62	0.54 ± 0.86	0.44 ± 0.73	0.72 ± 1.08	0.50
RDF	0.07 ± 0.13	0.06 ± 0.10	0.06 ± 0.12	0.07 ± 0.08	0.50
ARF	0.00 ± 0.00	0.01 ± 0.02	0.003 ± 0.01	0.004 ± 0.01	0.57
TTL	27.52 ± 22.21	32.67 ± 25.53	28.63 ± 22.17	33.15 ± 24.13	0.09
<b>Bottom (n = 10)</b>					
WC (m s <sup>-1</sup> )	0.94 ± 0.46	0.78 ± 0.41	0.79 ± 0.39	0.73 ± 0.35	0.12
FE	0.83 ± 0.06	0.74 ± 0.13	0.59 ± 0.23	0.46 ± 0.25	0.00
WV (m <sup>3</sup> )	6.53 ± 3.02	9.18 ± 4.12	13.43 ± 3.54	17.73 ± 3.46	0.00
OMV (cm <sup>3</sup> )	66.87 ± 30.46	88.33 ± 52.62	120.00 ± 56.57	140.50 ± 47.52	0.03
LDEN (ind m <sup>-3</sup> )					
NH	13.24 ± 9.48	14.28 ± 10.21	15.34 ± 11.84	14.31 ± 11.54	0.37
PREC	15.35 ± 18.49	17.78 ± 20.30	17.13 ± 20.30	19.15 ± 22.13	0.36
PRES	5.93 ± 3.84	6.86 ± 4.64	8.08 ± 5.47	6.44 ± 3.99	0.56
NRDF	1.39 ± 1.10	1.76 ± 0.96	1.55 ± 0.99	1.77 ± 1.21	0.20
RDF	0.77 ± 0.81	0.97 ± 1.09	1.28 ± 1.08	0.96 ± 0.90	0.21
ARF	1.48 ± 3.03	1.14 ± 1.73	1.25 ± 1.63	1.61 ± 2.18	0.08
TTL	39.63 ± 29.87	45.19 ± 32.26	45.84 ± 32.41	46.02 ± 33.08	0.22

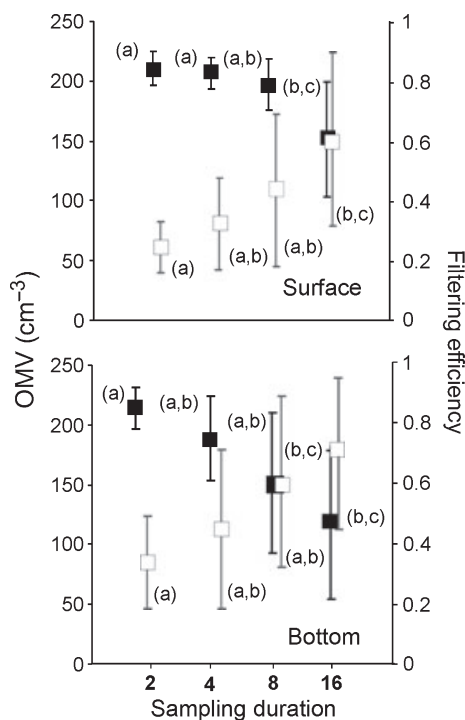


Fig. 2. Experiment 2: Mean and standard deviation for organic matter volumes (OMV, white) and filtering efficiency (black) for different sampling durations (minutes) in surface and bottom samples. Similar letters = treatments with no significant differences ( $P > 0.05$ ) according to Dunn's multiple comparisons

### Extrusion

The lack of differences in abundance of the smaller larvae (NH, PREC and PRES) between maximal and minimal velocities and durations (situations that represent the highest water pressure inside the net) support the lack of significant

extrusion. This could be attributed to the fact that their body depth (400–800  $\mu\text{m}$ ) is at least 30% greater than the mesh size. This seems to be in agreement with results found by Boltovskoy (1981), who stated that the probability of extrusion of planktonic organisms decreases significantly for those whose diameter is 25% greater than the net pore.

### Vertical distribution of ichthyoplankton

The lower surface densities found for the most developed silurid larvae (NRDF, RDF, ARF) are in accordance with previous reports for daylight sampling (Fuentes and Espinach Ros, 1998). However, the lack of differences in densities for all velocities and durations within each depth stratum encourages us to hypothesize that this trend, together with the similar one found for the smallest larvae (NH and PREC), is probably due to a bottom skewed vertical distribution and not to net avoidance or larval extrusion at the surface. The explanation of complex mechanisms regulating vertical distribution of ichthyoplankton in rivers, which involve phototactic response, buoyancy and diel rhythms of the larvae, as well as hydraulics and the context of turbidity of the river (Pavlov, 1994; de Graaf et al., 1999), is far from the scope of this paper. However, we hypothesize that the bottom distribution of newly hatched and post-flexion larvae during daylight hours is not driven by the same mechanisms. Poor or lack of buoyancy and a strictly planktonic condition in an hydraulic context probably explains the slightly skewed bottom distribution of less developed larvae (NH, PREC, PRES). As larval development proceeds, more control of the vertical position is acquired, but a negative phototactic response is elicited, particularly for silurids. This would explain our almost null daylight surface captures of post-flexion silurids (NRDF, RDF, ARF), which, as has been previously observed (Fuentes, 1998), show an active surface swimming in the river only at night.

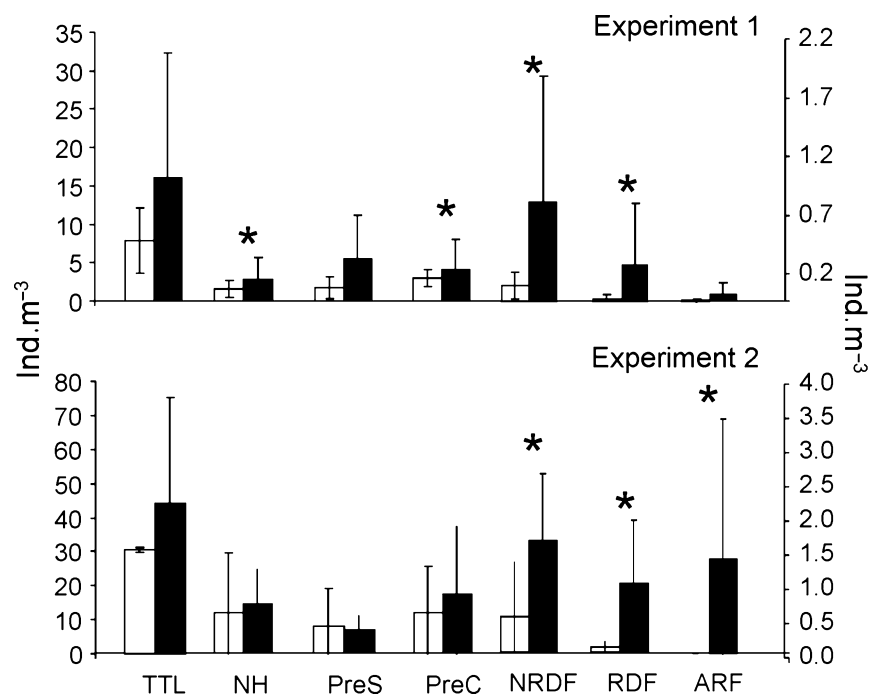


Fig. 3. Experiments 1 and 2: Mean larval abundance for each depth stratum and standard deviation for surface (white) and bottom (black) waters. TTL, NH, PRES and PREC: left axis; NRDF, RDF and ARF: right axis. \*Significant differences ( $P < 0.05$ ) between surface and bottom values according to Mann-Whitney test (Experiment 1) or Wilcoxon's test (Experiment 2)

#### Validity of inferences

It should be noted that our inferences on the net performance would be valid only in the context of this study (environment, sampling gear, range of treatments and groups of larvae studied). Working in marine environments, Thayer et al. (1983) and Hildén and Urho (1988) evaluated the performance of a Miller high speed sampler ( $2\text{--}7\text{ m s}^{-1}$ ) and a Gulf V sampler ( $0.9\text{--}3\text{ m s}^{-1}$ ) respectively, and concluded that evasion of large larvae (at low speed) and extrusion of small individuals (at high speed) were promoted. To our knowledge, there are no previous works on ichthyoplankton net performance in South American rivers where, in general, samplers work at a lower velocities than in marine environments. Such slow sampling velocities might impair larval fish extrusion and, at the same time, their potential effect on the net performance may be diminished by the high turbidities commonly found in the lower and middle sections of large rivers.

Our efforts to reduce the time elapsed between samples were probably not effective in preventing the variability in abundance within blocks. This short-time variability surely accounted for the lack of significant levels of differences between treatments. Under this circumstance, the possibility that some degree of evasive response of larvae (particularly of the oldest ones) at low sampling velocities, and extrusion of smaller larvae at high velocity and long duration samples, is not completely disregarded. Yet the lack of a trend in mean densities indicates that, if these phenomena occur, they account for a small part of total variability compared to the small time-scale and seasonal variations in larval abundance.

#### Recommendations

Further investigations involving more than one net-operator boat, the simultaneous deployment of nets with different mouth diameters and mesh sizes, and the application of treatments on the same larval patch are necessary to detect and quantify more subtle differences in capture rates obtained at different velocities and durations.

Finally, although limited by the intrinsic difficulties associated with the data, these results could be cautiously applicable in studies of South American river ichthyoplankton with similar conditions of water transparency and flow.

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- Author's address:** Carlos Mariano Fuentes. Instituto Nacional de Investigación y Desarrollo Pesquero, Paseo Victoria Ocampo No. 1 (B7602HSA), Mar del Plata, Buenos Aires, Argentina.  
E-mail: carlosmarianofuentes@gmail.com