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**Estimation of body weight of European sea bass (*Dicentrarchus labrax*) and Nile tilapia (*Oreochromis niloticus*) larvae by image analysis**

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**ABSTRACT:** The present study aimed to develop non-destructive methods to accurately follow the growth of fish larvae and post-larvae. European sea bass (*Dicentrarchus labrax*) aged 71-100 days post fertilization (dpf; 20-419 mg) and Nile tilapia (*Oreochromis niloticus*) aged 14 dpf (10-25 mg) were used as model species. Several measurements were performed on digital pictures of larvae to model body weight. Using body surface area, contour, length and depth, it was possible to estimate accurately the body weight of young European sea bass and Nile tilapia ( $r^2$  of 0.98 and 0.95, respectively).

**Keywords:** Image analysis; Body weight; Surface area; European sea bass; Nile tilapia

### Introduction

The early growth of fish can have a huge impact on their subsequent performance or traits during their entire life, for example as regards size, reproductive success or sex determination (Saillant et al, 2007; Doupe and Limbery, 2005; Baroiller et al, 2009; Vandeputte, 2012). Consequently, monitoring the growth of very small fish is of considerable interest. However, one of the main issues in measuring the growth of very small fish refers to the innocuousness of the weighing protocol, which requires removal of excess water. There is little precise information on the impact of this method, but it is supposed to be harmful to larvae (Krejszeff et al, 2013). Alternatively fish body weight (BW) can be inferred from the measurement of their body length from digital photographs after the calculation of mass-to-length relationship or more elaborated modelling of fish body shape, as it was shown in adult European sea bass *Dicentrarchus labrax* (Costa et al, 2013). In the latter article, fish BW was estimated more accurately using outline morphometry combined with multivariate techniques than on the basis of body length only. However, the accuracy of these indirect methods is often limited due to variable condition factors among individual fish, and it could be limited in early life stages due to a strong allometric growth patterns. The present study aimed at testing whether fish body weight can be predicted with a greater accuracy from digital pictures by measuring body dimensions other than body length only. Two fish species with a high economic importance, the European sea bass and the Nile tilapia were examined in the present study.

### Materials and Methods

**Biological material.** The fish used in this study were obtained from the spontaneous (for Nile tilapia) and artificial (for sea bass) reproduction of captive broodfish in the experimental infrastructures of GAMET (Groupe d'Aquaculture continentale Méditerranéenne et Tropicale, Montpellier, France) and Ifremer (Palavas-les-Flots, France), respectively.

**Measurements.** For European sea bass, the experiment was performed on five batches of larvae aged 71, 80, 87, 92 and 100 dpf (days post fertilization). At each age, 50 fish were sacrificed with an excess of anesthetic. BW was obtained using a precision scale (nearest 0.01 g) after careful drying with blotting paper, and standard body length was measured under a dissection microscope with a micrometer (nearest 0.1 mm). A digital picture of each fish was taken with a digital camera (12.2 Mpixel). For these pictures, each fish was placed on a light table (to increase the contrast) onto a numbered transparent plastic sheet, with a graduated (0.5 mm) ruler used as a reference.

For Nile tilapia, the experiment was conducted on 100 siblings aged 14 dpf (10-25 mg). The measurements of body weight and the capture of digital photographs were as for sea bass. Seven fish did not survive handling and were not taken into account in any analysis.

The following body dimensions were measured from digital photographs on the computer, using the ImageJ freeware (available at <http://rsb.info.nih.gov>; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD): standard body length (BL), maximal body depth (BD), body contour excluding fins (BC) and body surface excluding fins (BS). All four dimensions are given straight away by the software once the fish body has been contoured manually or automatically, which is feasible if it exhibits enough contrast with the background.

**Statistical analyses.** Phenotypic correlations between body dimensions and BW were estimated using the CORR procedure of SAS (Version 9.3, SAS Institute, Cary NC).

Multiple regression models were tested to evaluate the relevance of the different morphometric variables to predict BW with the REG procedure of SAS (Version 9.3,

SAS Institute, Cary NC). We used the coefficient of determination ( $r^2$ ) and the Akaike information criterion (AIC) to test the accuracy of BW estimation.

## Results and Discussion

**European sea bass.** Fish averaged 43 mg and 16 mm SL at the beginning of the experiment (71 dpf), and 246 mg and 26 mm SL at the end (100 dpf, Table 1). At 87 dpf, fish were size-graded in order to increase the number of observations on small fish. The phenotypic correlations between the morphometric dimensions ranged from 0.927 to 0.993. The best correlation with BW was obtained for body surface ( $\rho$  of 0.98). The model with the lowest AIC (1366) and the highest  $r^2$  (0.98) was the one including all the four morphometric variables (Table 2). This  $r^2$  value was similar to those found by Costa et al (2013) on large European sea bass (about 250g). Consequently, the BW of small sea bass can be estimated accurately from picture measurements using the following model:

$$BW = 160.85 - 14.94 * BC + 5.09 * BS - 131.43 * BL - 142.55 * BD$$

Yet, the use of a simple model (BW against BS) was still highly satisfactory (AIC = 1454,  $r^2 = 0.96$ ). The relevance of BS for the estimation of BW had been previously showed in adult Alaskan salmon (Balaban et al, 2010a).

**Table 1. Body weight (BW, mg) and dimensions of European sea bass and Nile tilapia larvae according to age (days post fertilization), Values are means  $\pm$  standard deviations.**

Age (dpf)	Sea bass					Nile tilapia
	71	80	87	92	100	14
Trait						
BW	43.1 $\pm 19.5$	148 $\pm 58.1$	236 $\pm 78.9$	204 $\pm 59.5$	246 $\pm 66.8$	15.1 $\pm 3.61$
BS	39.1 $\pm 9.53$	93.7 $\pm 21.3$	115 $\pm 25.4$	107 $\pm 19.3$	124 $\pm 20.9$	15.7 $\pm 2.30$
BC	37.4 $\pm 3.23$	58.1 $\pm 6.36$	64.2 $\pm 7.62$	58.0 $\pm 5.21$	62.4 $\pm 4.89$	2.08 $\pm 0.20$
BL	16.6 $\pm 1.41$	23.4 $\pm 2.51$	25.8 $\pm 2.89$	25.2 $\pm 2.26$	27.2 $\pm 2.25$	0.69 $\pm 0.19$
BD	3.57 $\pm 0.64$	5.64 $\pm 0.71$	6.11 $\pm 0.81$	5.89 $\pm 0.59$	6.37 $\pm 0.59$	0.49 $\pm 0.20$

BS: body surface (mm<sup>2</sup>); BC: body contour; BL: body length and BD: body depth (mm). N=50 at each age.

**Table 2. Coefficients of determination  $r^2$  and AIC values for different models of simple and multiple regressions to estimate BW.**

N° traits in the model	Trait(s)	Sea bass		Tilapia	
		$r^2$	AIC	$r^2$	AIC
1	BC	0.86	1784	0.57	164
1	BD	0.87	1768	0.01	242
2	BC + BD	0.89	1728	0.57	166
1	BL	0.93	1610	0.13	229
2	BC + BL	0.93	1608	0.59	162
2	BL + BD	0.93	1606	0.60	158
3	BC + BL + BD	0.93	1601	0.71	131
1	BS	0.96	1454	0.94	-22.9
2	BS + BD	0.97	1439	0.94	-21.6
2	BC + BS	0.97	1412	<b>0.95</b>	<b>-41.1</b>
2	BS + BL	0.97	1407	0.94	-21.4
3	BC + BS + BD	0.97	1404	0.95	-40.2
3	BC + BS + BL	0.97	1384	0.95	-40.0
3	BS + BL + BD	0.97	1380	0.94	-19.7
4	BC + BS + BL + BD	<b>0.98</b>	<b>1366</b>	0.95	-38.2

BS: body surface; BC: body contour; BL: body length and BD: body depth.

**Nile tilapia.** Tilapia weighed on average 15.1 mg at the time of the study (Table 1). BD was not significantly correlated with BW, BS and BC, but was significantly and negatively correlated with BL ( $\rho = -0.86$ ). BS and BC were significantly and positively correlated ( $\rho = 0.84$ ) and both were strongly correlated with BW ( $\rho = 0.97$  and  $0.75$ , respectively). The analyses and the estimations of the AIC and the coefficient of determination  $r^2$  revealed that the model with the best AIC (-41.1) was the one using only BS and BC to estimate BW. The model using all the four measurements showed a slightly lower AIC (-38.2) but a similar  $r^2$  value (0.95). Consequently, BW can be estimated accurately from picture measurements using the following model:

$$BW = -5.38 - 3.61 * BC + 175.35 * BS$$

According to these results, it seemed that body length was not always a reliable predictor of fish BW, as it was previously shown by Balaban et al (2010a, 2010b), and especially in very small fish. In addition, one major advantage to evaluate BW from the values of BS and BC is the fact that these two measurements can be obtained automatically from digital pictures.

## Conclusion

The higher relevance of BS in comparison to the other measurements (purely unidimensional morphometric variables) owes to its bidimensional nature. Although unidimensional, BC is influenced by both BL and BD.

The high coefficients of determination found in the present study on European sea bass as in Nile tilapia meant that when picture quality is sufficient, the body weight of fish larvae and small juveniles can be estimated accurately from digital pictures. The resolution of digital cameras already enables photographing several fish at the same time without compromising the accuracy of measurements. These capacities will just increase with future technological developments. This will enable the collection of much larger samples or reduce substantially the time of measurements in future experiments.

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