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Individual growth monitoring of European sea bass larvae by image analysis and microsatellite genotyping

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Abstract:

The aims of the present study were to develop non-lethal methods to identify individual fish larvae and post-larvae before tagging and accurately follow their growth characteristics. European sea bass (*Dicentrarchus labrax*) was used as a model species at four different ages ranging from 71 to 100 days post fertilization (dpf).

Two different methods were tested for non-lethal tissue sampling from each larva for DNA analysis: 1) using a sterile absorbent paper to sample mucus and/or epithelial cells by rubbing the fish skin and 2) fin-clip of the bottom part of the caudal fin. Whatever the age of the larvae, the genotyping rate (at 12 microsatellite markers) was low with the use of sterile absorbent paper but relatively high with fin-clip sampling at 80 and 87 dpf (on average 17 and 63% of the loci genotyped for sterile paper and fin clips, respectively).

Several measurements were performed on digital pictures of sea bass larvae to model body weight. Using area, perimeter, length, height and volume, it was possible to estimate body weight with a coefficient of determination $r^2 = 0.98$ on very small larvae (body weight ranging from 20.0 to 419.3 mg).

The present results suggested that individual monitoring of the growth of European sea bass larvae can be achieved by combining image analysis and microsatellite genotyping as early as 87 dpf or 236 mg mean body weight.

Highlights

► Development of an accurate method to follow growth characteristics on larvae ► Body weight was predicted from pictures with only the area and perimeter traits. ► Body weight prediction was accurate (coefficient of determination $r^2 = 0.98$). ► Individual identification was possible by fin clip genotyping on 200 mg fish.

Keywords: Individual identification ; Image analysis ; Body weight ; Surface area ; Perimeter ; European sea bass larvae

ABSTRACT

The aims of the present study were to develop non-lethal methods to identify individual fish larvae and post-larvae before tagging and accurately follow their growth characteristics. European sea bass (*Dicentrarchus labrax*) was used as a model species at four different ages ranging from 71 to 100 days post fertilization (dpf).

Two different methods were tested for non-lethal tissue sampling from each larva for DNA analysis: 1) using a sterile absorbent paper to sample mucus and/or epithelial cells by rubbing the fish skin and 2) fin-clip of the bottom part of the caudal fin. Whatever the age of the larvae, the genotyping rate (at 12 microsatellite markers) was low with the use of sterile absorbent paper but relatively high with fin-clip sampling at 80 and 87 dpf (on average 17 and 63 % of the loci genotyped for sterile paper and fin clips, respectively).

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Keywords: individual identification, image analysis, body weight, surface area, perimeter, European sea bass larvae

46 1. Introduction

47

48 The early growth of juvenile fish can have impact on key performance characteristics later in
49 life such as body weight (Saillant et al., 2007; Doupé and Limbery, 2005), survival (Diaz et
50 al., 2011), behavior (Nicieza and Metcalfe, 1999), reproductive success (Lee et al., 2012) or
51 sex determination (Saillant et al., 2003; Vandeputte, 2012). In the cichlid *Cichlasoma*
52 *citrinelum*, the largest juveniles become males whereas the smallest ones become females
53 (Francis and Barlow, 1993). Consequently, monitoring the growth performance of very small
54 fish is of considerable interest. However, weighing small fish is hazardous due to the need to
55 remove excess water with absorbent tissue to improve accuracy, but potentially at the cost of
56 a decrease in survival. There is no precise information available on the impact of this method
57 on fish survival and growth, but it is supposed to be harmful to larvae or very small fish.
58 Using image analysis could be a good way to reduce this problem and estimate the weight of
59 fish without manipulation. Several studies showed that the body weight or length could be
60 estimated using image analysis, as the structured light technology measuring the volume
61 (Storbeck and Daan, 1991) or the stereo-video technology measuring the length (Costa et al.,
62 2009; Shortis et al., 2013). In the same way, Costa et al. (2013a) demonstrated the utility of
63 using shape analysis of digital images to monitor body weight of adult European sea bass
64 *Dicentrarchus labrax* (around 250g). The weight of the fish was estimated with a coefficient
65 of determination $r^2 = 0.977$, higher than when using the log transformed body length ($r^2 =$
66 0.944), a more commonly used predictor. However, all these studies were performed on big
67 fish (body weight higher than around 200 g) and the application of these techniques to very
68 young fish faces a number of potential difficulties, as described by Costa et al (2009) showing
69 that the error of the weight estimation was inversely correlated by the size of the fish. The

accuracy of the prediction of the body weight using digital photograph measurements could be limited in early life stages due to a strong allometric growth.

Furthermore, to individually monitor the growth performance of small fish, it is necessary to identify each fish at the smallest possible size or youngest age. Individual identification can be done in fish with the use of physical tags, such as passive integrated transponder (PIT) tags (Prentice et al., 1990) or RFID microglass tags (Nanotec RFID, Lutronic International, Rodange, Luxembourg, www.nanotec.net; 6 mm long, 1 mm diameter, 10 mg weight), well described by Costa et al. (2013b). However, due to their size, the minimum tagging size was ranged between 300 and 450 mg in zebrafish (around 26 mm; Cousin et al., 2012) and in European sea bass (105 days post fertilization (dpf); 36 mm; Ferrari et al, in review). Identification methods using individual tags are not available for fish smaller than this size.

DNA genetic markers such as microsatellites or SNPs (Herbinger et al., 1995; Carleton et al., 2002; Trinh et al., 2013) have proved reliable for individual fish identification, but they need a relatively high quantity of sample tissue. Non-lethal tissue sampling of small fish that can provide sufficient DNA for downstream analysis is a particular challenge. Mirimin et al. (2011) have recently developed a method to collect DNA of Atlantic cod post-larvae by rubbing fishes with a sterile paper, but these authors used fish for which RFID microglass tags is potentially applicable (330-1610 mg, 43-52 mm).

The present study aimed to develop: i) a non-lethal method to identify individually fish larvae and post-larvae, and ii) whether it is possible to estimate the body weight (BW) of very small fish only from digital photograph measurements, and whether BW can be predicted with a greater accuracy by measuring other dimensions than body length only.

2. Material and Methods

2.1. Biological material

This study was carried out on European sea bass produced in March 2012 by artificial fertilization in a full factorial mating design between 5 dams and 30 sires from a West-Mediterranean population. Broodstock management and hormonal induction of spawning, artificial fertilization and incubation of eggs were done according to the protocols described in Saillant et al. (2001). Floating (live) eggs were separated from sinking (dead) ones 48 hours post fertilization by decantation at a salinity of 38 ‰ (Chatain, 1994), and introduced in equal proportion for each dam into a single tank to reduce environmental effects during the larval rearing. The rearing procedures that were used are the standards developed for the first three months described in Chatain (1994).

2.2. Non-lethal methods to individually identify larvae

2.2.1. Tissue sampling

Two different methods were used to obtain tissue samples from each larva, previously anaesthetised with MS_{222} (2 min at 0.07 $g.l^{-1}$). The first used sterile absorbent paper to sample mucus and/or epithelial cells by rubbing the fish skin as described in Mirimin et al. (2011). The second used a fin-clip of the lower part of the caudal fin stored in 70% EtOH. After sampling, larvae were maintained for five days in different tanks to estimate the mortality consecutive to each sampling method. The experiment was performed on five batches of larvae aged 71, 80, 87, 92 and 100 days old. At each age, 150 larvae were used among which 100 were tissue sampled, 50 with a sterile paper, 50 with a fin-clip, the remaining 50 being only anaesthetized to serve as controls for the anaesthetic procedure.

2.2.2. DNA extraction and genotyping

DNA was extracted from tissue samples using a proteinase K digestion, and the Chelex (Bio-Rad; www.bio-rad.com) extraction procedure. For each sample, 150 µl of 5 % Chelex solution, 15 µl of 1x Tris-EDTA buffer (10 mM Tris, 1mM EDTA) and 10 µl of proteinase K (10 $mg.ml^{-1}$) were added to the fin or the sterile paper, then incubated at 55°C for 2 hours, then at 96°C for 10 minutes. After that, the supernatant (containing DNA in aqueous solution) was transferred into a new clean tube. To verify the proper functioning of the extraction procedure, PCR was performed with sea bass specific primers (*amh* gene: 5'CCTAAGCTCCAGCTGACCAC 3' and 5' CTCCAACAGTGCAGGAGACA 3', D'Cotta, personal communication). Amplification was performed in a 20 µl PCR mixture containing 2 µl of DNA, 2 µl PCR buffer, 200 nmol of each primer, 0.4 µl of dNTPs mix and 0.11 µl of QiaGen Taq DNA Polymerase (ref 201207).

The following thermocycling procedure was used for PCR amplification (2 µl of DNA into 20 µl): denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 60°C for 45 sec and 72°C for 1 min. A final extension step was then performed at 72°C for 10 min. Negative (sterile water and sea water) and a positive control (sea bass purified genomic DNA) were included alongside each PCR run.

DNA for which the extraction procedure was a success, was precipitated with sodium acetate (Na-acetate, $1/10^{th}$ of the DNA solution volume) and 100% EtOH, (2 fold of the DNA solution volume). Samples were then centrifuged for 15 min at 12 000 g), and the supernatant removed. The pellet was washed with 1 ml of 70% EtOH, and a second centrifugation was performed (2 minutes at 12 000 g). The supernatant was discarded and the pellet dried. The DNA was resuspended with 50 µl of sterile water was performed.

To assess the possibility of identifying fishes, genotyping was performed by Labogena (Jouy en Josas, France) with a commercial suite of 12 microsatellites markers on 10 DNA samples obtained from sterile paper swabs and 10 DNA issued from fin of fishes at 71, 80 and 87 dpf.

2.3. Estimation of growth performances

At each sampling date, an additional 50 fish were sacrificed with an excess of anaesthetic (MS_{222}) to obtain the weight (after a careful drying with absorbent tissue) and length of each fish. Body weight (BW) was obtained using a precision scale (to the nearest 0.01 g), standard length (SL) was measured with a graduated ruler (to the nearest 0.1 mm) after magnification of the larvae. A digital picture (JPG format, 4272 x 2848 pixels) of each fish was taken using a stand with a Canon EOS 1100D digital camera (12.2 Mpixel). For these photos, each fish was placed on a numbered thin transparent plastic sheet to link the picture to the weight and length measurements, over a light table to increase the contrast, and beside a graduated ruler used as a reference.

Image analysis was performed with the ImageJ software (available at http://rsb.info.nih.gov; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). This software allows measurement of area, perimeter, length and height of each fish. The method allowed the use of fin-clipped animals without biais because it does not take into account the fish caudal fin in such measures. From length and height, the volume was also calculated as follows:

$$Volume = \pi * Length * Height * Height/6 \quad (1)$$

The different steps of image analysis (see Figure 1) were: i) converting the image to 8-bit (black and white), ii) adjusting the brightness and contrast to distinguish the larva clearly from

the background, iii) adjusting the threshold to blacken only the area of the larvae, iv) filling holes which may still exist, and v) analysing pixel particles. The ruler taken on each photo with the fish was used to convert all measurements from pixels to mm.

2.4. Statistical analyses

Statistical differences between survival rates between the tissue sampling methods and between genotyping results were performed with the NPAR1WAY procedure of SAS (Version 9.3, SAS Institute, Cary NC). Phenotypic correlations between the measurements done on the image those measured directly on the fish were estimated using the CORR procedure of SAS (Version 9.3, SAS Institute, Cary NC). Multiple regression models using length, height, perimeter, area and volume variables were tested using the REG procedure of SAS (Version 9.3, SAS Institute, Cary NC) to evaluate the efficiency of the different traits measured to predict BW. This last procedure estimated the coefficient of determination (r^2) and the Akaike information criterion (AIC) testing whether the BW is accurately estimated or not. To find the best model and regression equation to estimate BW, the PLS (Partial Least Square) procedure of SAS (Version 9.3, SAS Institute, Cary NC) was performed. The database was divided into two parts: 74 % of the measurements were used to predict the model and the remaining 26 % to validate it. In each age group, 13 larvae were randomly chosen to go into the validation dataset and the 37 other larvae were put into the dataset used for the prediction model.

3. Results

3.1. Estimation of growth performances

186 Fish were on average 43 mg weight and 16 mm length at the beginning of the experiment (71
187 dpf), and 246 mg weight and 26 mm length at the end (100 dpf, Table 1). The reduction in
188 size after 90 dpf was due to a grading that was operated to keep only the smallest fish to allow
189 the performance of more experiments on small size, the most interesting to set up an early
190 tagging and measurement method.

191 The phenotypic correlations between the measured traits were all high, ranging from 0.927 to
192 0.993, and the trait with the greatest correlation with BW was area (Table 2). The model with
193 the lowest AIC (1303.4) and greatest r^2 (0.980), was the one using all the image measures
194 (perimeter, length, area, height and volume, Table 3). In such a case (k=5), the PLS modelling
195 allowed estimating BW (with only 74 % of the data) with a r^2 of 0.981, and BW in the
196 validation group (26 % of the data) was estimated with a r^2 of 0.979. The model used was the
197 following:

198 $$BW = 133.497 + 3.335 * Area - 7.371 * Perimeter - 72.992 * Length - 280.594 *$$
199 $$Height + 217.072 * Volume \qquad (2)$$

200 The regression between measured and predicted values of BW with this model is shown in
201 Figure 2 for both prediction and validation datasets.

202

203 3.2. Tissue collection, DNA extraction and genotyping

204 The survival rate of control and sampled fish according to the age (71, 80, 87, 92 and 100
205 dpf), SL and BW is shown in Figure 3. Across all ages, the survival rate of the control larvae
206 was high, ranging from 98 to 100%. Except at 71 dpf, the survival rate of sampled fish was
207 also high (ranging from 90 to 100%) with a similar mean survival for fish sampled with
208 “sterile paper” (SP; 99%) and control fish (CO; 99%), slightly higher than the mean survival
209 of “fin-clipped” fish (FC; 93%). These differences were not significant except when CO or SP

survival rates were 100% whereas FC survival rate was 90% ($Chi^2 = 5.21$, $P = 0.02$ for FC *vs.* CO at 92 dpf, and for FC *vs.* SP at 87 and 92 dpf). At 71 dpf, the survival rate of CO fish was significantly greater than those of FC and SP fish ($Chi^2 = 40.4$, $P < 0.01$ for SP *vs.* CO, $Chi^2 = 26.3$, $P < 0.01$ for FC *vs.* CO, $Chi^2 = 2.5$, $P = 0.11$ for FC *vs.* SP). The survival rate generally increased with SL or BW. At 92 dpf, when fish were graded to keep the smallest fish, there was a reduction of the survival rate for the FC fish, lower than at 87 or 100 dpf.

The verification of the extraction procedure performed by PCR amplification of a sea bass specific gene showed that whatever the sampling procedure (fin-clip or sterile paper), the extraction method was functional, and the specific gene was amplified. Genotyping results (from 10 SP DNA and 10 FC DNA at 71, 80 and 87 dpf) showed differences between the sampling methods (Figure 4). SP fish were not or poorly genotyped whatever the age (genotyping rate, calculated as the percentage of genotyped microsatellites markers by the total number of microsatellites markers tested, ranging from 13 to 19%). Significantly fewer were genotyped ($Chi^2 = 6.08$, $P = 0.01$ with all ages combined) than FC fish (genotyping rate ranged from 2 to 77%). Deleting the data at 71 dpf for which the genotyping results were very low, the genotyping rates of FC and SP were 63 and 17%, respectively.

4. Discussion

An objective of this study was to develop a method to monitor growth performance of fish larvae using image analysis. Indeed, phenotypic correlations showed that body weight was highly correlated with some image measurements, r^2 ranging from 0.927 to 0.981. Furthermore, the value of the Akaike criterion estimated that the best model was the one including all image derived measurements. Consequently, it was possible to estimate body weight of small larvae with just measuring traits on a picture. With 74% of the dataset, the

model (1) allowed estimating BW with a coefficient of determination r^2 equal to 0.980 and an AIC of 1303.4. This model was then checked on 26 % of the dataset, and the estimated coefficient of determination r^2 was equal to 0.979.

These correlation coefficients were greater than those found by Costa et al. (2013a) on older European sea bass (average body weight of 254g or minimum area of 50 cm²). Indeed, with their model, these authors estimated a r^2 equal to 0.955 for 75 % of their dataset (used for class modelling) and 0.970 for their independent test (25 % of the dataset). In the present study, the high correlation coefficient meant that when the image quality is sufficient, the picture measurements were sufficient to accurately estimate the body weight of European sea bass larvae. Unlike Costa et al. (2013a), in the present study, image analyses excluded the fins, which are transparent at this stage and for which the weight is negligible. This small detail, which could considerably increase or decrease the area of the fish, explains probably why the correlation coefficient is higher in the present study than in Costa et al. (2013a).

We noticed an overestimation of the length of the fish on the picture measurement compared to the measured length (with a ruler). Indeed, comparing the estimated and the measured length of the larvae, an overestimation of the length of around 3.50 % was shown, probably due to a difference between the landmarks of the length. On bigger European sea bass, Costa et al. (2013a) developed an automatic approach usable in real-time to estimate BW with a specific image analysis protocol. A possibility to improve the present method would be to simplify the equation of BW estimation, taking into account only one criterion. Although the best model included estimated area, length, height, perimeter and volume of the fish, the simplest model showing highly satisfactory AIC corresponded to the model which takes into account only the area (AIC = 1454.4; r^2=0.963), which is, in addition, an easily automatable measure. Consequently, it is also possible to estimate quite accurately the body weight of sea bass larvae with just the area measured on the picture. In Alaskan salmon, Balaban et al.

(2010b) estimated body weight with area, length and width traits, and showed that the simplest model with just the fish area was sufficient to satisfactory estimate body weight without loss of accuracy; the addition of length and width not significantly improving the estimation. Furthermore, in the image analysis, caudal fins were erased manually for each fish, which is time consuming but also could reduce the accuracy of the measurements. With adult Alaskan pollock, Balaban et al. (2010a) estimated body weight based on the whole fish area, but also without fins, and without fins and tail, and concluded that their exclusion was not necessary to reliably estimate body weight. However, tail surface proportionally to fish surface seemed higher in larvae than in adult fishes, and including tail could be a problem especially in the case of fish which would be DNA sampled through tail clipping.

At 80 dpf, individual DNA sampling for genotyping fishes by fin clipping or by rubbing is possible without sacrificing larvae. The low survival rate at 71 dpf could be explained by the size of the fish but also by the fact that this was the first experiment we performed, and some details of anesthetic dosage or rubbing method were not optimized. To improve this technic or to develop it at a large scale, a rigorous preparation is a necessity for the survivability of the fishes.

DNA collection and extraction were possible with the clipping of a small piece of larval caudal fin (around 6 mm^2) but also with a rubbing of the larvae with sterile paper, as was previously shown with Atlantic cod weighing around 1230 mg (7 fold more than in the present study, Mirimin et al., 2011). While the DNA extraction was satisfactory, genotyping individuals was not possible in some cases as with sterile paper. This could be explained by a too small DNA quantity to allow efficient automated genotyping or by the extraction method which does not allow dosing the DNA quantity. Using DNA collection by fin clipping, the quality of genotyping results seems to be correlated with the age of the tested fishes (and probably with the size of the fin clip). The bigger the fish, the larger the fin piece for

284 extraction and genotyping. Consequently, it seemed possible to identify fish by microsatellite
285 analysis at 87 dpf using DNA collection by fin clipping, at an average weight of 236 mg. With
286 the present methods, genotyping was not practically possible using a standard parentage
287 assignment multiplex methodology with such very small DNA quantities, even if DNA
288 amplification was possible with the *amh* gene. The use of other DNA extraction methods used
289 for low-quantity DNA (less than 1 $ng.\mu l^{-1}$), such as those successfully applied with insect
290 legs, embryos or faeces (Arandjelovic et al., 2009; Watts et al., 2007; Zhan et al., 2008) may
291 be helpful to improve the genotyping success.

292 It will be interesting to study the caudal fin regrowth rate to estimate the minimal delay
293 between two samplings on the same individual (repeated genotyping being necessary to
294 identify each fish as long as individual tagging by e.g. micro glass tags is not possible).

295

296 5. Conclusion

297 Estimating body weight through image analysis was possible with adequate accuracy and
298 reliability ($r^2 = 0.98$), starting at 71 dpf or when larvae reached 43 mg mean body weight, 16
299 mm standard length. For individual identification through genotyping, the most efficient non-
300 lethal DNA sampling method was fin clipping. Regarding the individual identification, it was
301 clear that the "sterile paper" methodology was not suitable with small larvae, and with fin
302 clipping, the methodology used did not allow to genotype fishes before 87 dpf (or when the
303 larvae reached 200 mg mean body weight, 25 mm standard body length), probably due to a
304 lack of DNA material.

305

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

HdV, MV and BC contributed to the experimental design, data analysis, interpretation of data and manuscript preparation. EP contributed to the molecular analysis and MOD contributed to the experimental design and the data collection.

All authors approved the final version.

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408

409 **Table 1**

410 Growth performance of the larvae according to age (days post fertilization, dpf), expressed as
411 mean ± standard deviation. BW: body weight measured; SL: standard length measured; Area,
412 perimeter, length, height and volume were estimated with image analyses. N=50 at each age.
413 Only the smallest fish were kept after 87 dpf, explaining the decrease in weight between 87
414 and 92 dpf.

Age (dpf) Trait	71	80	87	92	100
BW (mg)	43.14±19.5	148.3±58.1	236.0±78.9	204.2±59.5	245.7±66.8
SL (mm)	15.9±1.51	21.9±2.31	25.5±2.85	24.7±2.29	26.4±2.26
Area (mm^2)	39.1±9.53	93.7±21.3	114.9±25.4	106.8±19.3	124.1±20.9
Perimeter (mm)	37.4±3.23	58.1±6.36	64.2±7.62	58.0±5.21	62.4±4.89
Length (mm)	16.6±1.41	23.4±2.51	25.8±2.89	25.2±2.26	27.2±2.25
Height (mm)	3.57±0.64	5.64±0.71	6.11±0.81	5.89±0.59	6.37±0.59
Volume (mm^3)	930±414	3243±1152	4217±1473	3748±1050	4722±1212

415

416

417 **Table 2**

418 Phenotypic correlations between all measurements at all the ages (N=250, *p*-value<0.001).

419 BW: body weight measured; SL: standard length measured.

Trait	SL	Area	Perimeter	Length	Height	Volume
BW	0.973	0.981	0.927	0.965	0.932	0.977
SL		0.986	0.953	0.990	0.945	0.954
Area			0.966	0.993	0.963	0.979
Perimeter				0.969	0.945	0.933
Length					0.954	0.962
Height						0.968

420

421

422 **Table 3**

423 Coefficient of determination r² and AIC (Akaike information criterion) for different models of

424 multiple regression to estimate body weight.

N° traits in the model	Trait	r^2	AIC
1	Perimeter	0.860	1784.1
1	Height	0.868	1768.4
2	Perimeter + Height	0.889	1728.2
1	Length	0.930	1609.5
2	Perimeter + Length	0.931	1608.4
2	Length + Height	0.932	1605.8
3	Perimeter + Length + Height	0.934	1601.1
1	Volume	0.954	1504.9
2	Perimeter + Volume	0.956	1496.5
2	Height + Volume	0.958	1489.0
2	Length + Volume	0.963	1457.0
1	Area	0.963	1454.4
3	Perimeter + Height + Volume	0.964	1453.0
3	Perimeter + Length + Volume	0.964	1451.6
2	Area + Height	0.965	1438.9
2	Perimeter + Area	0.969	1411.6
2	Area + Volume	0.969	1410.9
2	Area + Length	0.969	1407.4
3	Perimeter + Area + Height	0.970	1403.6
4	Perimeter + Length + Height + Volume	0.971	1398.6
3	Length + Height + Volume	0.971	1396.7
3	Area + Length + Volume	0.972	1390.1
3	Perimeter + Area + Length	0.972	1383.5
3	Perimeter + Area + Volume	0.973	1380.9
3	Area + Length + Height	0.973	1380.0
4	Perimeter + Area + Length + Volume	0.974	1369.4
4	Perimeter + Area + Length + Height	0.975	1365.5
3	Area + Height + Volume	0.978	1323.1
4	Perimeter + Area + Height + Volume	0.979	1315.9
4	Area + Length + Height + Volume	0.980	1306.3
5	Perimeter + Area + Length + Height + Volume	0.980	1303.4

425

426

427 Figure legends

428

429 Figure 1: Description of the four stages of the image analysis: a) original image, b) 8-bit
430 image, c) with an adjustment of brightness and contrast, and d) with an adjustment of the
431 threshold.

432

433 Figure 2: Regression between measured and predicted values of fish body weight with model
434 (1) for predicting (white triangles) and checking (black circles) datasets.

435

436 Figure 3: Survival rate according to the age (a), the standard length (b) and body weight (c) of
437 the larvae, and the sampling method (FC= fin clip fish, SP= sterile paper fish, CO= control
438 fish)

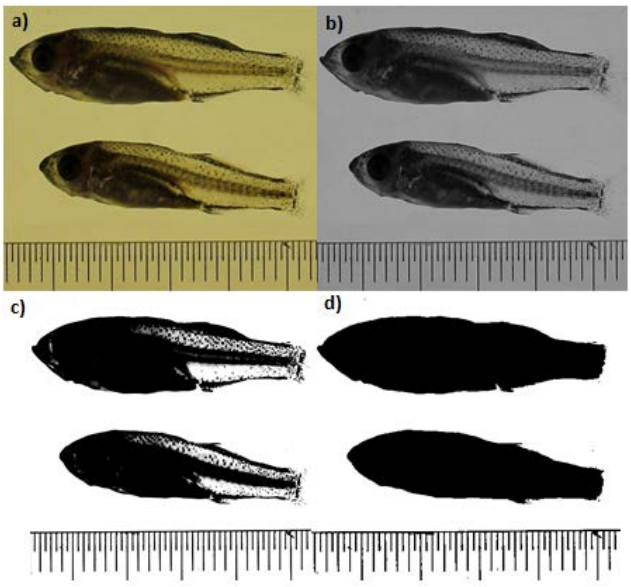
439

440 Figure 4: Number of genotyped microsatellite markers (out of 12) at 71, 80 and 87 days post-
441 fertilization (dpf) according to the DNA method of sampling: sterile paper (grey) or fin clip
442 (black).

443

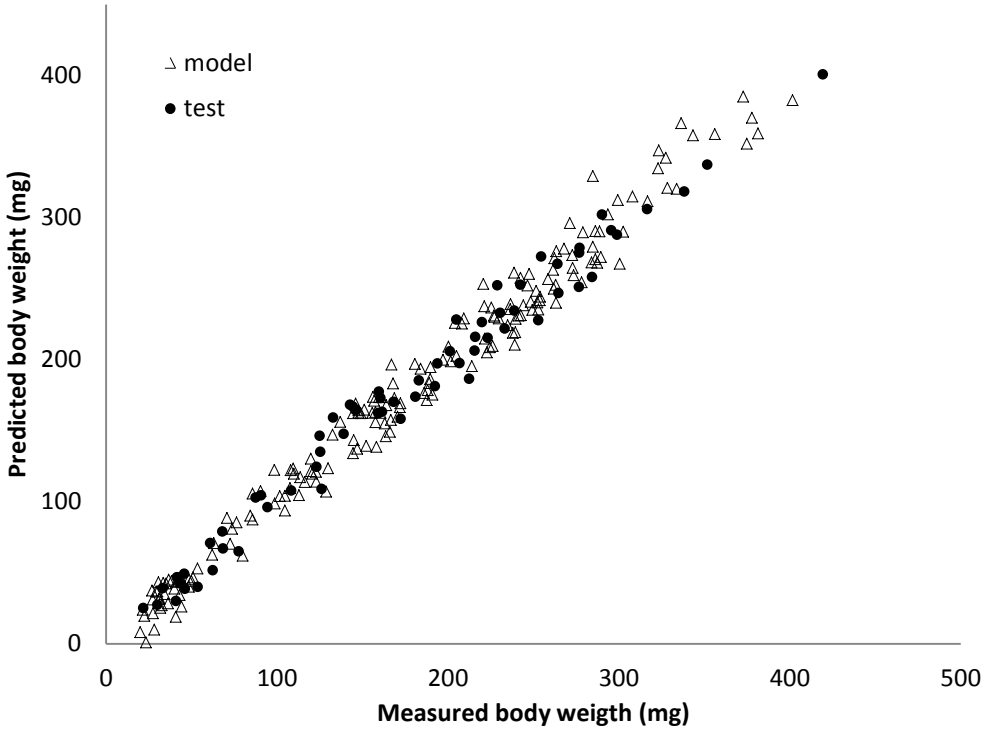
444

445 **Figure 1**



446

447 **Figure 2**



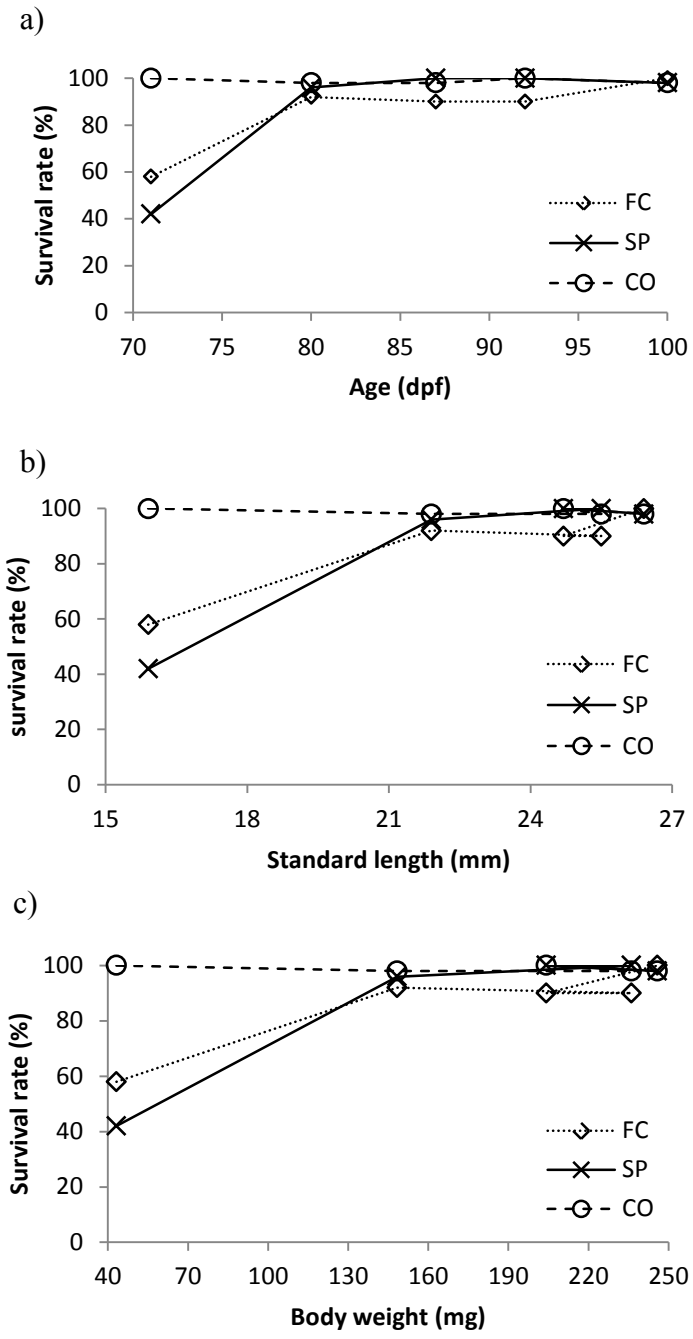
448

449

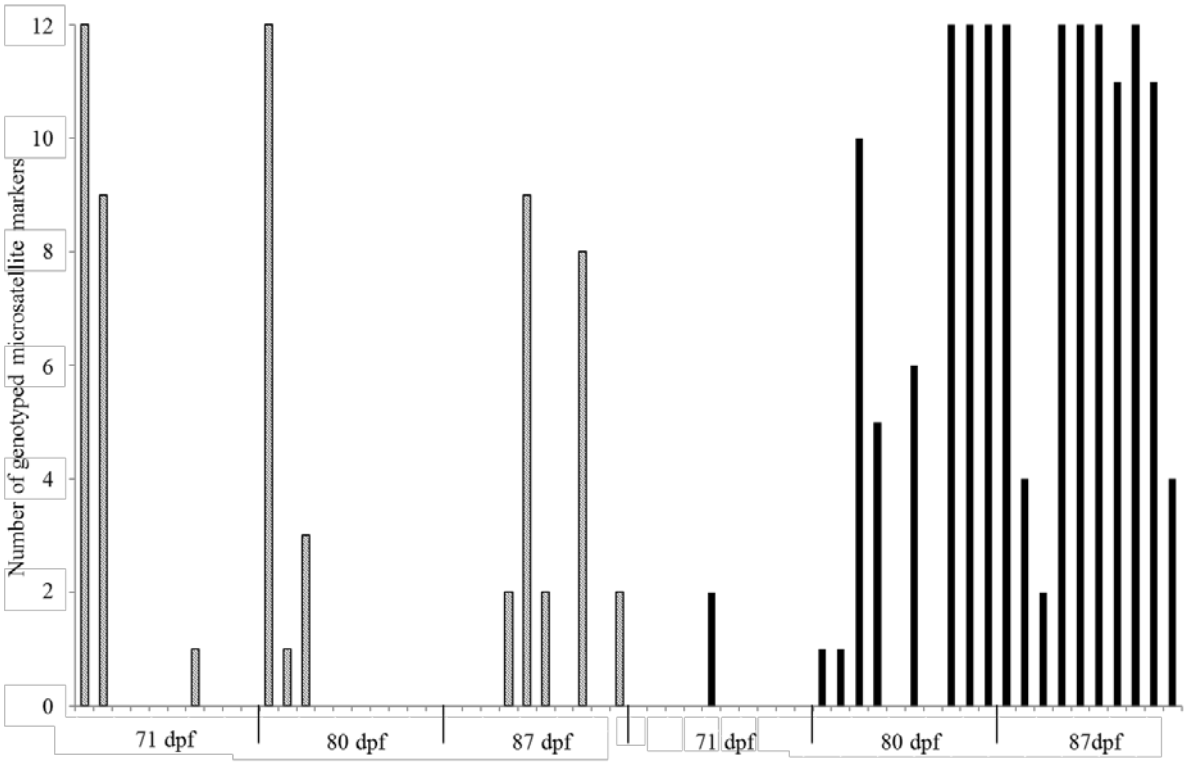
450

451

Figure 3


a)
Survival rate (%)
Age (dpf)
FC
SP
CO
b)
survival rate (%)
Standard length (mm)
FC
SP
CO
c)
Survival rate (%)
Body weight (mg)
FC
SP
CO

460 **Figure 4**



461

462