MARINE FISH EGG QUALITY INDICATORS IN AQUACULTURE, A REVIEW OF TECHNIQUES AND RESULTS

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Extended abstract

Background: Despite most of marine teleosts can produce large quantities of viable eggs in captivity, the quantity and quality of the larvae are low and variable, being the initial quality of the eggs one of the main causes of the variability. Considering that commercial hatcheries rely on good larval quality, the establishment of a series of criteria for egg quality is of paramount importance.

Objectives: This review presents an overview on some of recent researches carried out in IRTA and University of Salzburg (2004-2010) to address this bottleneck through correlating egg biochemical contents (BC) to embryo/larva success in a marine teleost considered as new species for Mediterranean aquaculture.

Target species: Besides production cost reduction and market enlargement, species diversification is one of the main strategies to ensure future expansion of aquaculture industry. A series of biological and commercial characteristics make the common dentex, *Dentex dentex*, a highly suitable species to enter mariculture systems as a new species to supplement the intensive sparids production and to increase diversification.

Applied methodologies: (i) Viable eggs were obtained from natural spawning of broodfish in captivity. (ii) Egg biometrical parameters (i.e., wet mass, dry mass, and water content) were recorded. (iii) Embryo/larva viability parameters (VPs, i.e., floating rate, hatching rate, and survival rate) were determined. (iv) Egg biochemical parameters (i.e., carbohydrate metabolites/enzymes, vitellogenin-derived proteins [VDP], non-Vtg-derived protein [non-VDP], free amino acid [FAA], proteinic amino acids [PAA], lipid classes [LC], fatty acids [FA], and morphological characteristics of lipid vesicle [LV]) were characterized and quantified. (v) A variety of statistical analyses were used to

define the relationships existing between the egg BCs (as egg quality indicators) and the VPs (as criteria for embryo/larva success) that are currently used in mariculture systems.

Results: Although a combination of statistical methods were used to correlate the egg BCs to embryo/larva success in common dentex, the current review was prepared based on the results of regression models. Carbohydrate (CH) metabolite contents and enzyme activity of the egg together with VDP, non-VDP, FAA, PAA, LC , and FA contents for one side and lipid vesicle (LV) morphological characteristics on the other were significantly and strongly correlated to embryo/larva success through almost 350 simple regression model (11 [r^2=0.184□0.490] for CHs and enzymes, 16 [r^2=0.095□0.634] for VDPs, 55 [r^2=0.079–0.637] for non-VDPs, 49 [r^2=0.605–0.875] for FAAs, 19 [r^2=0.919−0.991] for PAAs, 5 [r^2=0.459–0.739] for LCs, 201 [r^2=0.640–0.948] for FAs, and 10 [r^2=0.293–0.480] for LV).

Conclusions: (i) Under a biological/physiological perspective the significant relationships found between egg BCs and embryo/larva success in common dentex highlight the importance of egg composition during the initial events of embryonic/larval development. The results obtained present a series of new relationships (either in agreement or against previous findings) that deserve further investigation to define their physiological ground. (ii) Under a more applied perspective these relationships indicate a potential use of egg BCs, as bio-markers, to predict egg quality in aquaculture. These markers can also be used as molecular probes to assess the improvements in broodstock management.

Key words: *Dentex dentex, egg quality, embryo/larva success, mariculture*

INTRODUCTION

Although most of marine teleosts can produce large quantities of viable eggs in captivity, the quality of larvae is low and variable (Samaee, 2010 and references therein). The initial quality of eggs is accounted for as one of the main causes (Brooks et al., 1997). The review aim at to present an overview on the results obtained (both published and new findings [unpublished data]) by the authors of the current article to address this bottleneck through correlating common dentex, *Dentex dentex*, (a new candidate for Mediterranean aquaculture to increase the diversification in mariculture systems) egg biochemical contents –BC- (as egg quality indicators) to a series of viability parameters –VP- (as criteria for embryo/larva success) that are currently used in mariculture systems.

2. DETERMINATION OF EMBRYO/LARVA VPs

Common dentex eggs were obtained from natural spawning of broodfish (Giménez et al., 2006). Floating rate –FR- (the ratio of floating eggs to the total eggs collected) was promptly determined after collecting egg samples while hatching rate –HR- (the ratio of hatched eggs to the incubated eggs), and survival rate –SR- (the ratio of larvae that survived at a certain day post-hatch [dph] to the incubated eggs) after incubating egg samples into 96 well microplates (Shields et al., 1997).

3. EGG BCs IN RELATIONSHIP WITH EMBRYO/LARVA SUCCESS

3.1. Carbohydrate metabolites/enzymes

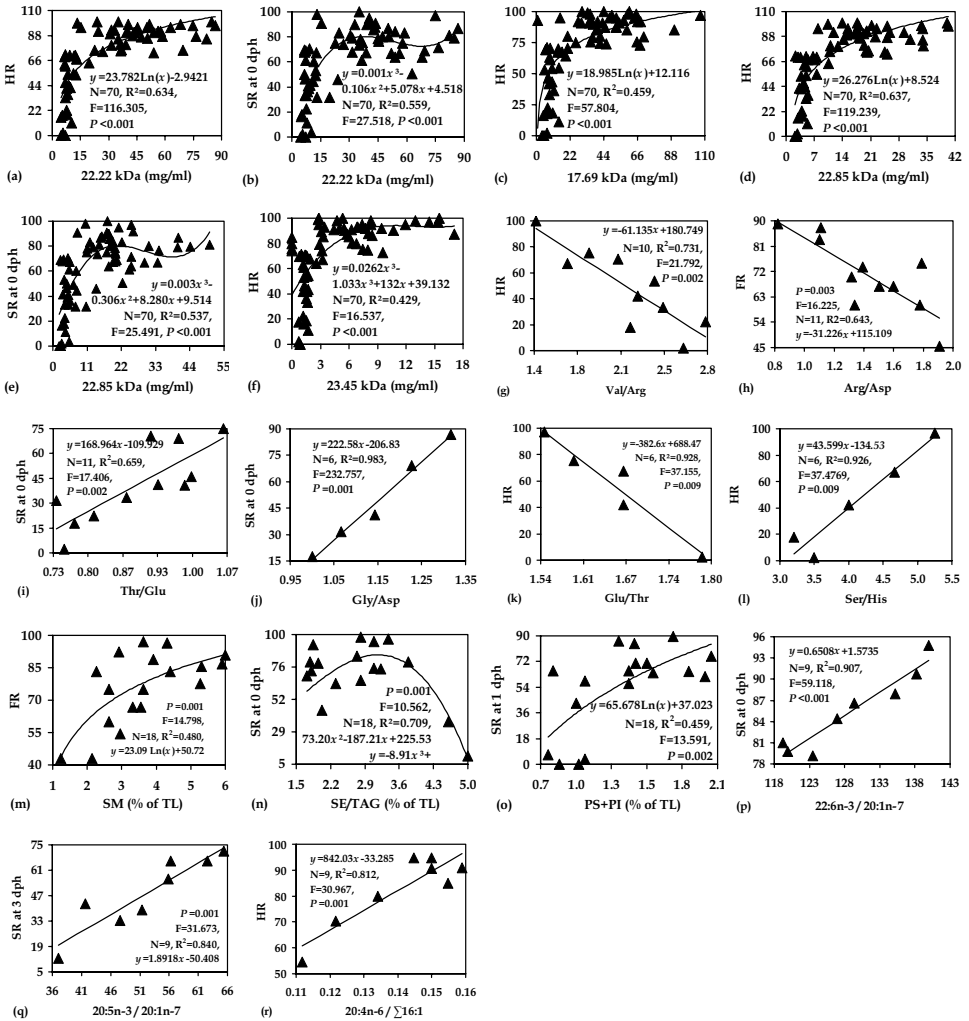
Spectrophotometry-based techniques were used to analyse metabolites and enzymes. The concentration of 4 metabolites (i.e., free 6-deoxyhexose, free monosaccharides, free ribose, and glucose) and 2 enzymes (i.e., pyruvate kinase and alkaline phosphatase) were found to be significantly linked to VPs through 11 simple regression models (SRM) with r^2=0.184□0.490 and p<0.001□p=0.053 (Giménez et al., 2006).

3.2. Egg yolk vitellogenin (Vtg)-derived proteins (VDP)

VDPs were purified (chromatography [Tsirogianni et al., 2004]), detected (SDS-PAGE), identified (LC-ESI-MS/MS), characterized (databank search), and quantified (densitometry). The concentrations of the 5 proteins (i.e., 77.97, 57.22, 17.69, 16.95 kDa [as VtgAb-derived proteins], and 22.22 kDa [as a VtgAa-derived protein]) were significantly correlated to VPs through 16 SRM (e.g., Fig. 1: a, b, and c) with r^2=0.095□0.634 and p<0.001□p=0.009 (Samaee et al., 2009a).

3.3. Egg non-vitellogenin-derived proteins (non-VDP)

Egg crude proteins were extracted, separated, and detected (SDS-PAGE). Then non-VDPs were characterized (specific-staining systems) and quantified (densitometry). The concentrations of 15 non-VDPs, with a frequency > 50%, (i.e., 105.43, 63.77, 50.48, 43.50, 33.59, 32.41, 31.40, 26.65, 25.56, 24.86, 24.01, 23.45, 22.85, 20.44, and 19.59 kDa) were significantly correlated to VPs through 55 SRMs (e.g., Fig. 1: d, e, and f) with r^2=0.079–0.637and p<0.001□p=0.001 (Samaee, 2010).



3.4. Egg free amino acids –FAA- (unbound amino acids)

FAAs were extracted, derivatised (Waters Pico-Tag method [Bidlingmeyer et al., 1986]), separated (HPLC), characterized (Spectra Physics SP4290 Integrator with WINner software), and quantified (Lyndon et al., 1993). The contents of 7 essential (i.e., arginine [Arg], valine [Val], isoleucine [Ile], leucine, tyrosine, threonine [Thr], and histidine [His]) and 8 non-essential (i.e., glutamic acid [Glu], asparagines, glutamine [Gln], aspartic acid [Asp], serine [Ser], glycine [Gly], proline, and cysteine) FAAs were found correlated with VPs through 49 SRMs (e.g., Fig. 1: g, h, and i) with r^2=0.605–0.875 and p<0.001 p=0.005(Samaee et al., 2010).

3.5. Egg proteinic amino acids –PAA- (bound amino acids)

Proteins were precipitated, and hydrolysed (incubation in acid [Finn et al., 1995]), and PAAs analysed as described for FAAs (Lyndon et al., 1993). The ratios of 7 essential (i.e., Thr, methionine [Met], Arg, Ile, Val, phenylalanine [Phe], and His) and 5 non-essential (i.e., Ala, Glu, Gly, Asp, and Ser) PAAs were correlated to VPs through 19 SRMs (e.g., Fig. 1: j, k, and l), with r^2=0.919−0.991 and p<0.001 p=0.009 (new findings [unpublished data]).

3.6. Egg lipid classes (LC)

After extraction of total lipid (Folch et al., 1957) LCs were separated, characterized (thin layer chromatography [Olsen and Henderson, 1989]), and quantified (densitometry [Henderson and Tocher, 1992]). Egg sphingomyelin (SM), phosphatidylcholin, phosphatidylserine (PS), phosphatidylinositol (PI), steryl+wax ester (SE+W), and triacylglycerol (TAG) contents were significantly correlated to VPs through 5 SRMs (e.g., Fig. 1: m, n, and o) with r^2=0.459–0.739 and p<0.001 p=0.002 (Samaee et al., 2009b).

3.7. Egg fatty acids (FA)

FAs were methylated (Christie, 1982), extracted (Tocher and Harvie, 1988), characterized and quantified (gas–liquid chromatography [Ackman, 1980]). The absolute concentrations, ratios, or combinations of 20 FAs (i.e., 14:0, 15:0, 16:0, 16:1n-7, 18:1n-7, 18:1n-9, 20:1n-9, 20:1n-7, 22:1n-11, 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:4n-6, 22:6n-3, and 24:1n-9) were significantly correlated to VPs through 201 SRMs with r^2=0.640–0.948 and p=0.0001–0.006 (Samaee et al., 2009b and new findings [unpublished data]).

3.8. Morphology of lipid vesicle (LV)

Since the shape of egg LV depends on its lipid composition, the characteristics of the egg factor is also considered in the review. Egg samples were photographed under a stereomicroscope at 25-fold magnification, pictures calibrated by means of micrometer scales, and the major and minor axes of the LV were measured. The ratio of the maximal to the minimal diameter of LV (RD) and lipid vesicle shape coefficient (SC), and embryo viability estimated from both RD and SC were significantly correlated to VPs through 10 SRMs with r^2=0.293–0.480 and p<0.001 (Lahnsteiner et al., 2008).

CONCLUSIONS

(1) Under basic aspects the significant and strong relationships found are addressing a crucial point of reproductive biology highlighting the importance of egg BCs during initial events of life in a marine pelagophil teleost such as common dentex. The results also present a series of new relationships (either in agreement or against previous findings) that deserve further investigation to define a physiological ground.

(2) Under applied aspects the efficiency of egg BCs to predict embryo/larva viability at critical stages of development indicates the potential use of egg parameters (that can be measured either in unfertilized eggs or at early developmental stages) as egg quality bio-markers that can also be used as molecular probes to assess improvements in broodstock management.

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