Aquaculture 362–363 (2012) 55–66

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

The selection of an ideal diet for *Ostrea edulis* (L.) broodstock conditioning (part B)

Ricardo González-Araya [a,b], Luc Lebrun [a], Claudie Quéré [c], René Robert [a,*]

[a] *Ifremer, Laboratoire de Physiologie et Ecophysiologie des Invertébrés Marins, Station Expérimentale d'Argenton et, Centre de Brest, Plouzané, France*
[b] *Universidad de Los Lagos, Departamento de Acuicultura y Recursos Acuáticos, Osorno, Chile*
[c] *Ifremer (LPI), Centre de Brest, Plouzané, France*

ARTICLE INFO

Article history:
Received 15 February 2012
Received in revised form 15 June 2012
Accepted 28 June 2012
Available online 7 July 2012

Keywords:
Ostrea edulis
Conditioning
Algal diets
Ingestion
Absorption
Biochemical composition

ABSTRACT

Four microalgae species (*Rhodomonas salina*, *Thalassiosira weissflogii*, *Thalassiosira pseudonana* and *Pavlova lutheri*) were evaluated to estimate their potential as food for *Ostrea edulis* (L.) reproductive conditioning. Best ingestion and absorption were observed with *R. salina* (3.44 and 1.59 mg g^{-1} h^{-1}, respectively), followed by *T. pseudonana* (2.75 and 0.98 mg g^{-1} h^{-1}) and *P. lutheri* (2.40 and 0.91 mg g^{-1} h^{-1}). Oysters fed *T. weissflogii* exhibited the lowest ingestion and absorption values (1.40 and 0.68 mg g^{-1} h^{-1}). Proximate composition (proteins and carbohydrates) and lipid content (fatty acids and sterols) analysed in four main tissues (gonad, digestive gland, muscle and gills) also differed significantly with diet. Protein ranged from 355 mg g^{-1} in the gonad of oysters fed *P. lutheri* to 837 mg g^{-1} in gills of oysters fed *T. weissflogii*; whereas carbohydrates ranged from 17.5 mg g^{-1} in gills of oysters fed *P. lutheri* to 271 mg g^{-1} in gonads of oysters fed *R. salina*. An overall poor enrichment in total PUFAs across all diets masked some of their potential impact on nutrition. In gonad, however, the major polyunsaturated fatty acids (polar lipid fraction) were EPA (≈19% for oysters fed *T. weissflogii* and 14% for those fed *P. lutheri*) and DHA (17% for oysters fed *P. lutheri* and 15% for those fed *R. salina*). Sterol contents showed a clear transfer from food to oyster tissues except with *P. lutheri*, from which neither methylpavlovol nor ethylpavlovol (characteristic of Pavlophyceae) were detected in oyster tissues. Histological analysis showed that gametogenesis was active in oysters fed *R. salina* and *T. weissflogii*, whereas only low gonadic development occurred in unfed oysters or those fed *P. lutheri*. *R. salina* is accordingly highly recommended for *O. edulis* broodstock conditioning whereas *P. lutheri* should be excluded.

1. Introduction

Broodstock conditioning is a key step in the process of rearing bivalves under standardized conditions. Its success has often been estimated in terms of the quality of bivalve eggs and larvae produced. Thus, initial egg lipid contents have been found to be positively correlated with either larval survival (*e.g., Mercenaria mercenaria* and *Crassostrea virginica*: Gallager and Mann, 1986; *Pecten maximus*: Le Pennec et al., 1991) or larval growth (*Ostrea edulis*: Helm et al., 1973). Although temperature has been considered to be the main environmental factor regulating bivalve reproduction (*e.g., C. gigas*: Fabioux et al., 2005; *Mytilus galloprovincialis*: Fearman and Moltschaniwskyj, 2010); feeding (i.e., the amount of food supplied) also seems to be an important factor for increasing fecundity (*e.g., C. gigas*: Chavez-Villalba et al., 2003; *Argopecten purpuratus*: Martinez et al., 2000a, 2000b). In contrast, the influence of the relative food value of different phytoplankton species (nutritional quality) on mollusc gonadic development has been very little explored, especially in *O. edulis*. The pioneer works of Frolov and Pankov (1992) and Millican and Helm (1994) provided relevant information in this field but only a few studies (Berntsson et al., 1997) have been carried out since. Indeed the appearance in France in the 1970-1980s of epizooties of *Marteilia refringens* (Comps, 1970) and *Bonamia ostreae* (Comps et al., 1980) and their progressive extension throughout Europe (see review in Laing et al., 2005) led to the collapse of *O. edulis* culture and research then focused more effort on recently introduced species (*e.g., C. gigas*: Helm and Millican, 1977; Robert et al., 1982; and *R. philippinarum*: Helm, 1990; Utting and Spencer, 1991).

In Europe, particularly France and Spain, *O. edulis* remains an emblematic species and attempts to develop "resistant strains" have been made in both countries (Lallias et al., 2010; Montes et al., 2003; Naciri-Graven et al., 1988). Moreover, such interest in flat oyster cultivation is now increasing in France due to high *C. gigas* juvenile mortalities (Pernet et al., 2010; Samain and Mc Combie, 2008).

To allow *O. edulis* genetic improvement through selection, the reliability of hatchery methods for this species needs to be improved. As already pointed out, conditioning is an important step in hatchery production of molluscs and particular attention needs to be paid to flat oyster feeding during this stage because hatchery-conditioned broodstock has been found to have lower fecundity than wild stock (Helm et al., 1991). We had already made an initial study to look for an ideal diet for *O. edulis* (González-Araya et al., 2010). This

* Corresponding author at: Ecloserie Expérimentale d'Argenton, 11 Presqu'île du Vivier, 29840 Argenton en Landunvez, France. Tel.: +33 2 98 89 29 42; fax: +33 2 98 89 29 59.
E-mail address: Rene.Robert@ifremer.fr (R. Robert).

0044-8486/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2012.06.029

work compared four monospecific microalgal diets based on ecophysiological and biochemical approaches, and assumed that the best microalgae should be those that were highly ingested, digested, assimilated and efficiently allocated to the reproductive compartment. The present study was designed to provide complementary information by testing the influence of four more microalgae on *O. edulis* consumption, ingestion, assimilation and reproduction.

2. Material and methods

The techniques used in this study were previously detailed in González-Araya et al. (2010), so only a brief outline will be given here.

2.1. Experimental design

In August 2008, *O. edulis* aged 18 months (≈5 cm length and 0.5 g flesh dry weight), originating from Bay of Quiberon (South Brittany, France) were submersed, at 5 m depth, for 1 month, in mesh bags tied to trestles in the Bay of Brest. They were then returned to the quarantine area of the Argenton hatchery, where they were maintained at 14 °C for an additional month, during which they were treated for a week with chloramphenicol at 8 mg l^{-1} to limit any development of vibrios. Thereafter, seawater temperature was increased by 1 °C weekly and, at beginning of October 2008, the flat oysters were transferred to translucent 50-l tanks where they were distributed homogeneously (30 oysters per tank, corresponding to an equivalent biomass of ≈1 kg total weight and 16 g dry flesh weight). During this preconditioning period oysters were fed a mixed diet of T. Iso and *Chaetoceros gracilis* used routinely in Argenton to feed most of mollusc at different stages of development (Ben Kheder et al., 2010). Triplicate tanks were set up for each of the four single diet species tested here. Oysters were maintained in a flow-through system at 19 °C and fed constantly at 900 $\mu m^3\ \mu l^{-1}$ or unfed (only receiving continuously 1 μm-filtered-seawater). Four different microalgae were tested as mono-specific diets at the same biovolume (measured daily and accordingly including variation in cell volume): *Rhodomonas salina* (mean volumetric size 160 μm^3, mean dry weight 130 pg $cell^{-1}$, strain CCAP 978/24), *Thalassiosira weissflogii* (900 μm^3, 250 pg $cell^{-1}$, strain CCAP 1085/1), *Thalassiosira pseudonana* (40 μm^3, 35 pg $cell^{-1}$, strain CCAP 1085/3) and *Pavlova lutheri* (40 μm^3, 20 pg $cell^{-1}$, strain CCAP 931/1). The choice of these species was based on their frequency of utilization in different mollusc commercial hatcheries worldwide (Borowitzka, 1997; Robert and Trintignac, 1996). Ingestion and absorption of the different microalgae were studied according to Beiras et al. (1994) over six consecutive weeks. It was hypothesized that the

Table 1
Fatty acid and sterol composition of total lipids of *Rhodomonas salina, Thalassiosira weissflogii, Thalassiosira pseudonana* and *Pavlova lutheri* expressed in mean relative content (weight % of total polar fatty acids ± S.D., n = 3). Values within the same line with a commun superscript letter are not significantly different at p = 0.05.

	Oyster diets							
Fatty acid	*R. salina*		*T. weissflogii*		*T. pseudonana*		*P. lutheri*	
14:0	7.26	(2.88)	7.86	(1.23)	6.76	(0.89)	10.04	(0.63)
16:0	13.60	(2.23)	13.60	(0.88)	24.05	(0.22)	19.45	(2.20)
18:0	0.54	(0.23)	0.00	(0.00)	0.00	(0.03)	0.43	(0.10)
16:1(n-9)	[a] 1.12	(0.22)	[b] 0.00	(0.00)	[b] 0.00	(0.00)	[b] 0.00	(0.00)
16:1(n-7)	0.74	(0.36)	20.14	(3.25)	17.30	(0.10)	16.27	(4.21)
18:1(n-9)	1.26	(0.56)	0.00	(0.00)	0.99	(0.04)	1.31	(0.33)
18:1(n-7)	2.04	(0.32)	1.18	(0.08)	1.24	(0.08)	1.72	(0.03)
16:2(n-4)	[a] 0.09	(0.01)	[b] 5.85	(0.90)	[ab] 2.21	(0.02)	[a] 0.48	(0.06)
16:3(n-4)	[a] 0.00	(0.05)	[b] 17.95	(1.44)	[c] 5.39	(0.10)	[d] 0.09	(0.01)
18:2(n-6)	[a] 18.04	(4.03)	[b] 0.57	(0.01)	[b] 1.25	(0.02)	[b] 2.42	(0.30)
18:3(n-6)	3.76	(1.61)	0.27	(0.01)	2.56	(0.02)	1.72	(0.27)
18:3(n-3)	[a] 11.51	(2.46)	[b] 0.59	(0.09)	[b] 0.13	(0.01)	[ab] 1.57	(0.44)
18:4(n-3)	[a] 13.66	(1.58)	[b] 1.54	(0.01)	[ab] 5.23	(0.45)	[ab] 6.62	(0.55)
20:4(n-6)	[a] 2.41	(0.20)	[b] 0.22	(0.01)	[c] 9.41	(0.01)	0.46	(0.35)
20:5(n-3)	9.51	(0.85)	20.43	(4.56)	14.70	(0.89)	23.37	(2.08)
22:5(n-6)	0.20	(0.21)	0.00	(0.00)	0.00	(0.00)	1.01	(0.26)
22:6(n-3)	8.18	(2.64)	3.60	(0.55)	4.64	(0.03)	10.75	(0.39)
TO.MONO	8.35	(1.29)	22.47	(5.89)	19.67	(0.28)	19.86	(4.27)
TO.(n-9)	2.48	(0.67)	0.09	(0.01)	0.99	(0.16)	1.37	(0.11)
TO.(n-7)	3.13	(0.29)	21.58	(2.22)	18.55	(0.08)	18.14	(4.22)
TO.POLY	68.74	(6.28)	52.86	(6.33)	47.08	(0.75)	49.41	(2.87)
TO.(n-4)	[a] 0.09	(0.40)	[b] 23.80	(1.57)	[c] 7.60	(0.10)	[ad] 0.57	(0.07)
TO.(n-6)	[a] 24.55	(5.49)	[b] 1.05	(0.26)	[ab] 13.45	(0.04)	[ab] 5.94	(0.77)
TO.(n-3)	44.02	(4.07)	26.35	(2.62)	24.71	(1.15)	42.79	(2.26)
(n-3)/(n-6)	[a] 1.79	(0.57)	[b] 24.97	(2.81)	[a] 1.84	(1.36)	[a] 7.21	(0.49)
22:6/20:5	0.86	(0.31)	0.18	(0.01)	0.32	(0.01)	0.46	(0.03)
22:5/20:4	[a] 0.08	(0.14)	[a] 0.00	(0.00)	[a] 0.00	(0.00)	[b] 2.19	(0.55)
fg $cell^{-1}$	26081.65	(13746.85)	5874.23	(708.67)	7593.36	(562.47)	2672.66	(636.59)
Sterols								
Brassicasterol	[a] 97.26	(0.45)	–	–	–	–	–	–
Cholesterol	[a] 0.36	(0.32)	[b] 4.36	(0.77)	[b] 6.10	(0.08)	[a] 0.40	(0.06)
Campesterol	[a] 0.28	(0.18)	[ab] 3.20	(1.11)	[b] 6.96	(0.05)	[ab] 2.90	(0.07)
24-Methylen-cholesterol	[a] 2.10	(0.31)	[b] 78.60	(1.10)	[b] 79.46	(0.34)	–	–
Stigmasterol	–	–	–	–	–	–	[a] 16.43	(1.95)
Isofucosterol	–	–	[a] 11.20	(0.80)	[b] 3.97	(0.14)	–	–
Methylporifera	–	–	–	–	–	–	[a] 13.80	(0.89)
β-sitosterol	–	–	–	–	–	–	[a] 11.58	(1.41)
Fucosterol	–	–	[a] 2.96	(0. 14)	[a] 3.52	(0. 06)	–	–
Desmosterol	–	–	–	–	–	–	[a] 1.57	(0.11)
Methylpavlovol	–	–	–	–	–	–	[a] 36.08	(1.90)
Ethylpavlovol	–	–	–	–	–	–	[a] 16.43	(1.44)
fg $cell^{-1}$	434.26	(193.97)	1685.47	(449.12)	135.04	(36.74)	806.16	(57.64)