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Recent advances in reproductional aspects of *Dentex dentex*

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SUMMARY – The common dentex (*Dentex dentex*) is a highly valued table fish in the Mediterranean region and elsewhere in the tropics, and is of great potential for mariculture. A basic requirement of intensive farming of any fish species is the ability to control fully the reproductive cycle and spawning in order to ensure a constant supply of high quality eggs and fry. However, basic knowledge on the reproductive biology of common dentex is extremely limited and, therefore, control of its reproduction in captivity poses a bottleneck in the establishment of its commercial culture. Between 1996 and 1998, research (funded by EU, FAIR-CT-95-0407) has been undertaken to provide the scientific and technical basis for effective and reliable reproduction of common dentex. Results on the reproductive biology, hormonal and environmental control of spawning, and broodstock management towards the development of a new species for Mediterranean mariculture are presented herewith.

Key words: Common dentex, hormones, photoperiod, reproduction.

RESUME – *"Progrès récents concernant les aspects de reproduction de* Dentex dentex*". Le denté* Dentex dentex *est un poisson méditerranéen et subtropical très estimé et de grand intérêt pour l'aquaculture. L'une des bases de l'élevage intensif des poissons est le contrôle de la reproduction et de la ponte afin d'assurer un approvisionnement constant et de qualité en œufs et alevins. Chez le denté les connaissances dans ce domaine étant restreintes, la maîtrise de sa reproduction est encore un des facteurs limitant son élevage. Entre 1996 et 1998, des recherches (EU FAIR-CT-95-0407) ont été entreprises pour répondre à cette demande. Les résultats relatifs à la biologie de la reproduction, au contrôle hormonal et environnemental de la ponte, à la gestion des stocks de géniteurs sont présentés ci-après.*

Mots-clés :*Denté commun, hormones, photopériode, reproduction.*

Introduction

Among the sparids, the common dentex (*Dentex dentex*) is a highly valued table fish in the Mediterranean region and elsewhere in the tropics and has been established as a prime new candidate aquaculture species over the last decade. Rearing experiments on juveniles have shown that common dentex express high growth rates and the ability to spawn spontaneously, being, therefore, highly suitable for intensive aquaculture. This species has been scarcely investigated, with most of the relevant studies being referred on its distribution (Bayle-Sempere *et al*., 1991), growth and feeding (Bibiloni *et al*., 1993; Riera *et al*., 1993; Efthimiou *et al*., 1994; Tibaldi *et al*., 1996) and larval development (Glamuzina *et al*., 1989; Franicevic, 1991; Kentouri *et al*., 1992; Koumoundouros, 1988). Studies on the reproductive biology of the common dentex are also limited and conflicted. D'Ancona (1950) concluded that *D. dentex* is a gonochoristic sparid (14 fish studied). In a brief presentation, Bauchot and Hureau (1986) mentioned that common dentex would be gonochoristic while some specimens could be hermaphrodite. Glamuzina *et al*. (1989, number of fish not cited) suggested protandric hermaphroditism of this species. In a recent study, Morales-Nin and Moranta (1997) indicated (macroscopic examination of gonads from 210 fish caught from the waters of Mallorca) an equal sex distribution and a possible gonochorism of common dentex.

Control of reproduction is essential for successful fish farming and, as for any aquaculture species, a thorough knowledge on the reproductive biology of common dentex provides the basis on its propagation in captivity. Between 1996 and 1998, research (funded by the EU, FAIR-CT-95-0407) has been undertaken to provide the scientific and technical basis for effective and reliable reproduction of this prime new species for mariculture. The main objectives have been: (i) to provide basic knowledge on its reproductive biology; (ii) to investigate environmental factors likely to be used for manipulation of spawning period; (iii) to develop an appropriate and cost-effective method for synchronisation of spawning and increasing yields and quality of gametes; and (iv) to support the development of a new candidate species for Mediterranean mariculture. The present work describes the results obtained on major aspects of the reproductive biology of common dentex maintained under intensive culture conditions.

Reproductive biology of common dentex

Sexuality pattern and sexual cycles

Sexual differentiation in common dentex occurs between 5 and 12 months of age. The relative frequency of both sexes does not differ significantly from 50%, although variations as a function of the age and the cohort of the fish could be observed. Among the 323 individuals studied, 321 had unambiguously either testis or ovaries. One 10 month-old fish had oocytes at the primary growth phase, but some cells seemed to be primary spermatocytes, and one 12 month-old fish had testis with spermatozoa present in the deferent ducts, but some oocytes at the primary growth phase were scattered in the tubules. Thus, results indicated that common dentex is a gonochoristic fish. Five stages of maturity have been defined for females (F1: oogenesis; F2: primary oocyte growth; F3: cortical alveolus stage; F4: vitellogenesis; F5: final oocyte maturation), and males (M1: spermatogenesis; M2: spermiogenesis I; M3: spermiogenesis II; M4: spermiation; M5: post-spawning) (Table 1).

Table 1. Classification of maturity stages in female and male common dentex

	Maturity stage	Histological features
Female		
F1	Oogenesis	Oogonia and oocytes at the first stage of meiotic prophase. Some scarce previtellogenin oocytes
F2	Primary oocyte growth	Oocytes with a homogenous vacuole-free cytoplasm, a circular nuclear membrane and 2-14 small spherical, peripherally-located nucleoli
F3	Cortical alveolus stage	Oocytes with peripheral cytoplasm-containing cortical alveoli, a lobated nuclear membrane with several nucleoli. Follicular envelopes and zona radiata well-visible
F4	Vitellogenesis	Oocytes with accumulated spherical, brown, yolk granules in the whole cytoplasm. Zona radiata thick and yellow
F5	Final maturation	Presence of hydrated large oocytes, with a peripheral germinal vesicle. Presence also of post-ovulatory follicles
Male		
M1	Spermatogenesis	A and B spermatogonia and presence or not of primary spermatocytes
M2	Spermiogenesis I	Presence of spermatogonia, spermatocytes, spermatids and spermatozoa
M3	Spermiogenesis II	Abundant spermatozoa in the testis and present in the deferent ducts. Some spermatids and spermatocytes also present
M4	Spermiation	Only spermatozoa and resting spermatogonia. Deferent ducts filled with spermatozoa. Milt could be obtained by stripping
M5	Post-spawning	Regressed testis. Albuginea was thick and the interstitial tissue abundant. Some phagocytosed spermatozoa present in the lumen of tubules and of deferent ducts. In some cases, the lumen of the tubules was nearly absent

The reproductive cycle of female common dentex comprises a long resting period, a short maturing – pre-spawning period and a breeding period (Fig. 1). The resting period lasts from June until January and at that time the ovaries contain mainly oocytes at primary growth (F2 females). During this period, a discrete spermatogenetic activity resumes more or less rapidly in males (October-December). The second phase lasts from February until March and oocytes at the cortical alveolus and vitellogenesis stage can be distinguished in the ovaries. The testis contain spermatids and spermatozoa. This period is characterized by an increase in gonad weight and gonadosomatic index (GSI); between the end of February and the end of June, an increase in GSI is found (peaked in May) followed by a sharp decrease (males: less than 0.2%; females: about 1%), regardless fish age. In both sexes the mean maximal weight of the gonads increase with age, reaching 70-75 g at 46 month-old fish, while the mean GSI reached 6 and 5% in the males and females respectively. Puberty in both sexes is reached near the second year of life. During the spawning period, about 100% of the males release sperm, while the percentage of mature females is a function of age: 30-70% of 2 year-olds; 50-80% of 3 year-olds; 70-100% of 3 year-olds. Under natural conditions common dentex in Crete (latitude 35°N) spawns over an 8 to 10-week period from April to June, under an increasing photoperiod (12:30-14:40 h light/day) and temperature (15.7 to 22.0°C). The spawning peak occurs in May under a temperature range of 17.1 to 21.5°C. In general males began spermiation up to two months before the spawning period of the female fish.

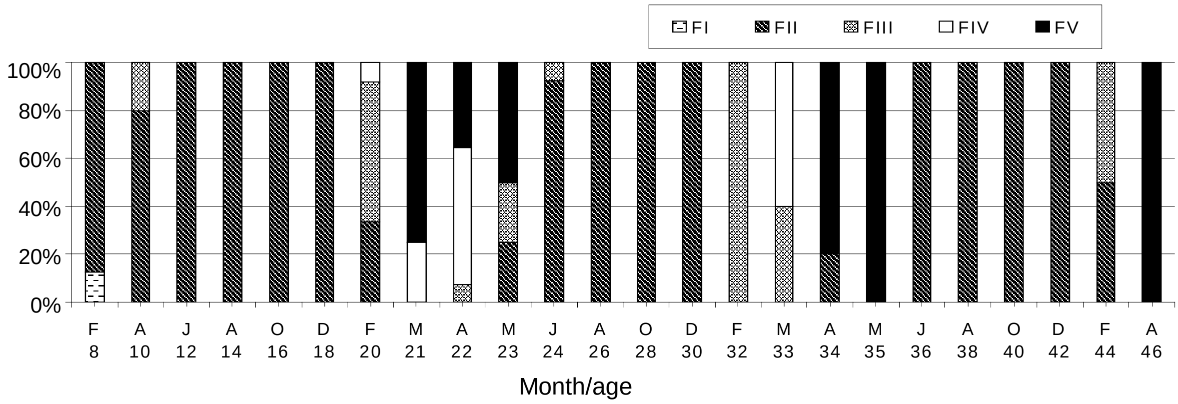

Fig. 1. Sexual cycle of female common dentex maintained under intensive culture conditions (% of maturity stages).

Common dentex is a batch spawner, i.e. it spawns multiple batches of pelagic eggs within a single spawning period. Concomitant to the maturation and ovulation of the first batch, populations (clutches) of oocytes undergoing vitellogenesis, cortical alveolus or primary oocyte growth stages are present. Recruitment of the successive clutches occurs directly from a large heterogeneous population of smaller oocytes. Common dentex of 0.9-2.5 kg give 4 to 18 separate clutches of eggs during one spawning period, with mean ovulatory rhythms of 30-70 h, and with a total annual fecundity of 114,000 to 607,000 eggs per female per year. Eggs are around 1 mm (range 0.925-1.050 µm) in diameter, transparent, contain a single oil globule, and tend to float at the surface of the tank.

Hormonal control of the reproductive cycle

Seasonal fluctuations and maturity stages related differences in serum 17β-oestradiol (E_2), testosterone (T), 11 ketotestosterone (11-KT), vitellogenin (VTG) and thyroid hormones (T_3 and T_4) are found during the annual reproductive cycle of common dentex. In females (Fig. 2), baseline T and E_2 values (<0.11 ng/ml) and negligible VTG concentrations are found during the post-spawning and pre-gametogenesis period (June to December), increase thereafter to reach a peak during the spawning period (mean ± SEM, T: 0.99 ± 0.21 ng/ml; E_2: 0.75 ± 0.16 ng/ml; VTG: 1313 ± 236 µg/ml). Thus, maximum values are found during vitellogenesis and spawning concomitant with a dramatic increase in GSI and oocyte diameter. Serum T, T_3 and T_4 exhibit a bimodal pattern of changes during the 2nd and 3rd reproductive cycle, with the first peak in August or October (F2 females) and the second around spawning period. No difference occurs in T and E_2 levels among maturity stages F3, F4 or F5; however, these are all significantly higher than those in stage F2. Serum VTG and T_3 concentrations were significantly higher in F4 and F5 than at the other stages. These findings are similar to patterns described in other marine teleosts (*Sparus aurata*, Kadmon *et al*., 1985; *Dicentrarchus labrax*, Prat *et al*., 1990; *Pagrus auratus*, Carragher and Pankhurst, 1993; *Pagrus pagrus*, Fostier *et al*., 1996; Kokokiris, 1998) and they support the widely held hypothesis for the role of E_2 in initiating and maintaining vitellogenesis. In addition, the high serum E_2 levels during spawning can be accounted for by production of this steroid from the remaining groups of vitellogenic follicles, as it is to be expected in a multiple spawner like common dentex.

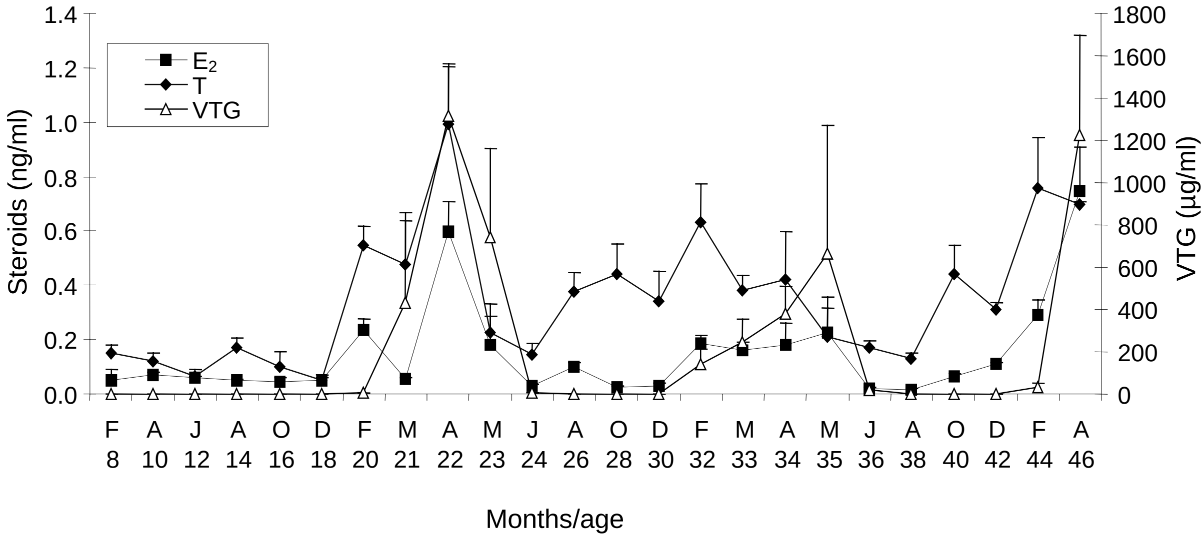

Fig. 2. Seasonal changes (mean ± SEM) in serum testosterone (T), 17β-oestradiol (E_2), and vitellogenin (VTG) levels in female common dentex during the 1st, 2nd, and 3rd reproductive cycle.

In males, as in females, seasonal changes in serum levels of the androgens T and 11-KT were well correlated with gonadal development. The presence of males that have completed spermiogenesis (M3) in December coincided with the surge of both androgens and this increase lasted until the end of the spawning period. The maximal plasma concentrations of 11-KT and T were 3.61 ± 1.19 ng/ml and 4.04 ± 1.19 ng/ml, respectively. Serum 11-KT and T levels were higher in M3 and M4 fish, while no significant differences were observed in serum T_3 levels among the maturity stages. Thus, results were in accordance with the established role of androgens in initiating and maintaining testicular development. However, no specific function for each steroid could be elucidated.

Energetics of gonad formation

Seasonal variations in serum and tissue levels of lipids in fish are related fundamentally to the metabolic costs of growth and to the reproductive cycle. In female common dentex, a general pattern, with maximum serum concentrations of total proteins (TP), total lipids (TL), and cholesterol (Chol) during pre-spawning – spawning period (F4 and F5 fish) was observed in all reproductive cycles. In addition, mature females (F5) had statistically higher TP, TL and Chol levels than mature males (M4). The total energy content (TE) of the female gonads displayed a similar seasonal pattern to that of GSI and it was positively correlated with maturity stage (stage F2: 22 kJ/g dw – F5: 27 kJ/g dw). The TE of the liver increased with increasing GSI until it decreased significantly some months before the spawning period. The energy density of the male gonads showed no clear correlation with the maturity stage or season. The TE of muscle increased from 22.8 kJ/g dw (F1) to 24.9 kJ/g dw (F3), and then decreased to 23.7 kJ/g dw, at stage F5. No relationship between the TE of the muscle in males and the TE of the liver in both sexes and maturity stage was found. In both sexes, no statistical difference in the lipid content of the gonads between the maturity stages was observed. The lipid content of the muscle increased from maturity stage 1 to maturity stage 3 and then decreased to a minimum value at stage 4 (males) or 5 (females). Thus, the pattern was similar to that found in the energy densities of the muscle.

Fish tissue lipids are generally characterised by their high content of long-chain polyunsaturated (PUFA), especially n-3 series fatty acids (FA). Fish are unable to synthesize PUFA, therefore, the dietary PUFA are essential for well-being and successful reproduction. In common dentex, the main n-3 PUFA found were docosahexaenoic (DHA, 22:6n-3) and eicosapentaenoic (EPA, 20:5n-3) acids. The main n-6 PUFA were linoleic (18:2n-6) and arachidonic (20:4n-6) acids. Palmitic (16:0) and oleic (18:1n-9) acids were the main fatty acids of the saturated fatty acids and monoenes, respectively. No statistical difference in the FA composition of muscle and liver between sexes was found. The FA composition of male gonad differed statistically from FA composition of female gonads by its high amount of saturated FA in both lipid fractions (triglyceride-phospholipid) and oleic acid in phospholipid fraction. The liver in comparison to muscle contained higher amount of saturated fatty acid, especially palmitic acid, and lower amount of PUFA in both lipid fractions. The triglyceride–phospholipid (TG/PL) ratio was 3 to 4 fold higher (1.8) in the muscle than in the liver (0.4) and the gonads (0.5). A clear seasonal pattern was found in palmitic acid percentages in TG fraction, in total saturated FA in TG fraction, and in palmitic acid, total PUFA and docosahexaenoic acid in PL fraction, with minimum values in February (both immature and mature fish) and maximum in summer. The opposite pattern was seen in the percentages of oleic acid in TG fraction, and linoleic acid in both lipid fractions and oleic acid and eicosapentaenoic acid in PL fraction and TG/PL ratio.

Collectively, these data indicate that female common dentex invest more than 2.5 times greater energy into the gonads than the male fish, and that during the period of the fast gonadal growth energy is transferred from female somatic tissue, especially from the muscle, to the gonads. Noticeable seasonal changes occur in the fatty acid pattern of dentex tissues, but since similar changes are found in tissues of both immature and mature fish, it seams that temperature and/or food, not reproductive stage, are the major factors affecting the fatty acid composition.

Hormonal induction of spawning

Common dentex breed spontaneously in captivity, however hormonal induction techniques were applied and checked for synchronization of spawning and increasing yields of gametes. Results showed that GnRHa-containing implants are an effective way to induce final oocyte maturation and ovulation in captive females, and increased egg production by more than 10-fold. In the males, these GnRHa implants had only marginal effect on sperm production and except of 11-KT did not induce dramatic changes in the plasma levels of the measured steroids. Pimozide had no discernible effect on ovulation success or milt volume, or on plasma hormone levels in either females or males. In addition, administration of an acute injection of GnRHa clearly stimulated steroid production. The effect on plasma levels of free 17,20β-P and E_2 was rapid (3 and 6 hours respectively) and sharp, whereas the effects on plasma levels of 17,20β,21-P were gradual and more prolonged. Plasma levels of free and conjugated 17,20β-P and 17,20β,21-P remained elevated in plasma even after 24 hours. Levels of 17,20β-P appeared to rise in controls also at 6 hours (around 7 ng/ml).

The two known maturation inducing steroids (MIS) in teleosts are 17,20-dihydroxy-4-pregnen-3-one (17,20β-P) and 17,20,21-trihydroxy-4-pregnen-3-one (17,20β,21P) (Nagahama, 1987; Thomas and Trant, 1989). We have also found that common dentex ovarian follicles during the ovulatory period have the potential for producing both steroids. 17,21-P is not still present, and 21-hydroxylase could be the limiting enzyme for the synthesis of 17,20β,21-P. It seems that 17,20β,21-P is the maturation inducing steroid in common dentex, but can be quickly metabolised after synthesis and action on the oocytes, in relation to the multiple ovulation pattern of this species for which the ovary contains, during the ovulatory period, both vitellogenic and maturing oocytes.

Environmental control of the reproductive cycle

Intensive fish farming procedures require a year-round egg production. Photoperiod and temperature are the main environmental factors used to extend (accelerate or delay) the spawning period in a variety of species (Devauchelle, 1984; Bromage and Cumaranatunga, 1988; Pavlidis *et al*., 1992; Carrillo *et al*., 1995; Zohar *et al*., 1995). Out-of-season spawning can be achieved by the use of constant light regimes, compressed photoperiods, or phase-shifted 12-month long cycles in phase or not with water temperature cycles. In order to investigate the effect of photoperiod on spawning time and on the associated changes in serum levels of sex steroids and vitellogenin, two groups of common dentex were exposed to phase-shifted 12-month long photoperiod cycles advanced (Group C) or delayed (Group B) by 3 months from the norm and constant temperature conditions (19.4 ± 0.9°C). A third group (Group A) served as controls (simulated natural photoperiod and ambient water temperature).

Results clearly showed that the rates of maturation and timing of spawning in the common dentex can be modified by thermo- and photoperiodic change without the need for hormonal induction. The controls spawned in April-June at the same time as fish in tanks under ambient day-length. Group C fish spawned up to 4 months in advance (December-April) to the control fish. In addition, a prolongation of spawning period was observed (130 days vs 63 days in control). In contrast, exposure of fish to protocol B delayed maturation and spawning period by 2 months (June-August), with little difference in the spawning spread (44 vs 63 days in control group). The quality of spawning (percentage of viable eggs), the spawning spread, relative fecundity (number of eggs/kg post-spawning female body weight) and spawning index (number of spawnings per female) of the experimental groups were higher or similar than the controls. Thus, by modifying photoperiod and keeping temperature constant (at an optimum range for spawning) enabled viable eggs to be obtained for a period of 9 months (December to August).

Changes in the timing of maturation and spawning among groups were reflected to the serum levels of sex steroids and vitellogenin. Regardless of photothermal manipulation and sex, maximal plasma steroid levels were detected during spawning (March, December, and June for control, advanced and delayed treatment respectively), and were of similar magnitude. The displacement of the spawning period, as well as the number of spawning females was correlated with the seasonal profile and magnitude of plasma vitellogenin.

Broodstock management

When broodstock management programmes were started in 1990's, common dentex broodfish originated from the wild (acclimatisation and domestication of wild adults or rearing of 0^+- and 1^+-year-old juveniles until mature stage). Since 1993, broodstock obtained from fish reared and bred in captivity have been developed and organized to provide the initial zootechnical basis for further genetic selection. Broodstock management is based on the successful manipulation of water quality, feeding regime and diet, thermo- and photoperiod cycle, adequate organization (size, density, sex ratio) of broodfish and prevention of stress.

Broodstock are usually kept in floating net cages and transferred to on-land spawning tanks about 4-6 weeks before spawning. However, stocks programmed for out-of-season egg production are held in indoor isolated units in order to be undisturbed during maturation and spawning. Spawning tanks, are commonly (Mediterranean area) circular, rectangular or sub-square of 10-20 m^3. Tanks are supplied with well-aerated seawater (salinity 35-37‰, pH 7.6-8.5) and equipped with outlets for automatic evacuation of sediments and for egg collectors, as well as systems for filtering, light and temperature control. The strategy of water renovation is almost the same for all sparids. It ranges between 100-150% per hour for the period following spawning. In the Eastern Mediterranean, with high summer temperatures, this condition is of major importance to avoid micro parasitism (Cryptocarion, Oodinium) and illness. But it is limited to 25-30% per hour during the 2-4 months of spawning season when the flow rate of water in the egg collector may be a mechanical stress for the eggs. Temperature control may be achieved by the use of a heat exchanger and/or by pumping water from a deep well (assuring constant temperature of 19-20°C all-year round). Artificial lighting is controlled either by astral timers (365-d model, latidude 35°N), or by fixed photophases (classical 24 hour switch on-off programs).

Common dentex broodstock is fed, at present, only with frozen raw fish or squid (after vitamin enrichment) distributed manually *ad libitum* twice or three times per week. Conversion index, biological and economical, is between 1.54-4.63% and 3.86-11.09% respectively until the fish are in spawning condition, at which time an associated loss of conversion efficiency is observed (average FCIb: 28.62%). No statistically significant difference is observed in the daily feeding rate (DFR) among fish in pre-spawning and spawning period (pre-spawning period: 0.44-2.51%; spawning period: 0.58-1.54%), while a significant increase of feeding occurs the two months following spawning (DFR: 1.04-2.27%).

Common dentex used as broodstock are 3-6 years old and, in general, an equal number of each sexes is distributed in the spawning tanks at densities of 10-15 kg/m^3. Around the spawning period, sex can be determined by visual criteria due to a marked difference in male and female coloring which become evident as spawning approached: males took on an intense bright color whilst the females are more pallid with pink dots. Alternatively, light stripping or biopsy of animals can be used. The sexual organization of brood stocks is an important factor of spawning success. The best spawning quality, in terms of percentage of viable eggs, and higher relative fecundity (i.e. number of eggs/kg post-spawning female body weight) is observed in the 1:1 sex ratio (respectively 51%, 604,492 eggs per kg). An important finding of the study is that steroid levels can easily be monitored in water taken from spawning tanks, and that differences in the ratios of males to females clearly affects steroid levels present in the water. Higher 11-KT, a steroid which is predominant in males, levels are found if more males are present and conversely higher 17,20β-P, the predominant steroid in females, if more females are present in the spawning tanks. In addition, spawning condition of the broodstock is reflected in the steroid levels present in the water. Thus, differences in conjugated 17,20β-P and 17,20β,21-P levels in water only occurred during the breeding season and not during the rest of the year. Level of conjugated 17,20β-P and 17,20β,21-P reach a maximum during 14:00 and 16:00 which is 1 to 5 hours prior to the spawning of eggs (spawning usually occurs between 17:00 and 21:00).

In conclusion, basic knowledge on the reproductive biology (timing of sex differentiation, sexuality pattern, sexual cycles, and the endocrine/metabolic changes associated with gonadal development) of common dentex have been obtained, providing the basis on its propagation in captivity. By manipulating photoperiod it is possible to alter spawning and produce eggs for at least 9 months of the year. In addition, first data on broodstock management have been obtained. Further studies on the development of appropriate diets to reduce dependence on raw food and on the identification of broodstock nutritional requirements for increased spawning quality are some future prospects.

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