The reproductive cycle of female Ballan wrasse *Labrus bergylta* in high latitude, temperate waters

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This 2 year study examined the reproductive cycle of wild female Ballan wrasse *Labrus bergylta* in western Norway as a precursor to captive breeding trials. Light microscopy of ovarian histology was used to stage gonad maturity and enzyme-linked immuno-absorbent assay (ELISA) to measure plasma concentrations of the sex steroids testosterone (T) and 17β-oestradiol (E$_2$). Ovarian recrudescence began in late autumn to early winter with the growth of previtellogenic oocytes and the formation of cortical alveoli. Vitellogenic oocytes developed from January to June and ovaries containing postovulatory follicles (POF) were present between May and June. These POF occurred simultaneously among other late maturity stage oocytes. Plasma steroid concentration and organo-somatic indices increased over winter and spring. Maximal (mean ± s.e.) values of plasma T (0.95 ± 0.26 ng ml^{-1}), E$_2$ (1.75 ± 0.43 ng ml^{-1}) and gonado-somatic index (I_G; 10.71 ± 0.81) occurred in April and May and decreased greatly in July when only postspawned fish with atretic ovaries occurred. Evidence indicates that *L. bergylta* are group-synchronous multiple spawners with spawning occurring in spring and peaking in May. A short resting period may occur between late summer and autumn when previtellogenic oocytes predominate and steroid levels are minimal. © 2010 The Authors

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INTRODUCTION

Ballan wrasse *Labrus bergylta* Ascanius are common inhabitants on rocky reefs along the Atlantic coasts of Morocco and Europe, ranging as far north as Trondheim in Norway (Quignard & Pras, 1986). Female fish exist in harems within the reproductive territory of a dominant male with which they will mate, spawning their benthic eggs over temporary nests (Hilldén, 1984). Like many other temperate wrasses, *L. bergylta* have proven effective cleaners of sea lice *Lepeophtheirus salmonis* from farmed Atlantic salmon *Salmo salar* L. These ectoparasites represent a serious economic and animal welfare liability to the European *S. salar* farming industry. Ulcerative

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wounds inflicted by sea lice often cause osmoregulatory stress and can lead to mass loss and, in severe cases, even death. In addition, infected farmed salmonids have been implicated as a contamination vector of wild populations (Bjørn & Finstad, 2002; Frazer, 2009). Chemical delousing treatments often used within the *S. salar* industry have received negative attention and vary in efficacy due to resistance development (Pike & Wadsworth, 1999; Tully & McFadden, 2000; Fallang *et al.*, 2004). Alternatively, biological control of sea lice is considered environmentally safe and its application is not subject to resistance problems. *Labrus bergylta* are the largest of the European wrasses attaining a maximum size of *c*. 60 cm (Quignard & Pras, 1986) and are therefore of sufficient size to keep in sea cages with larger 3–5 kg *S. salar*. At present, there is a growing interest in farming *L. bergylta* to reduce fishing pressure on local wild stocks and to provide a consistent supply to *S. salar* farmers. Such an approach should decrease the use and environmental impact of chemical delousing agents.

It is advantageous to understand the reproductive cycle of a species in an effort to co-ordinate effective captive breeding trials. Only limited information exists on the reproductive physiology of *L. bergylta* and, in particular, its seasonality in Norway. Teleost reproduction is often a highly seasonal event, especially at high latitudes due to the reproductive strategy of external fertilization. The resulting larvae are generally poorly developed at hatch and are highly susceptible to environmental fluctuations. Mature fish, therefore, perceive seasonal cues to help direct the timing of gonad production and breeding (Crim, 1982; Lam, 1983; Bye, 1989; Taranger *et al.*, 1998). These environmental signals are modulated *via* the brain-pituitary-gonad (BPG) axis to effect a co-ordinated endocrine cascade that results in the development of mature gametes (Bromage *et al.*, 2001; Okuzawa, 2002). Oocyte development can be classified into discrete categories according to morphological developments (Wallace & Selman, 1981; Tyler & Sumpter, 1996; Patiño & Sullivan, 2002). These changes are clearly visible using histological analysis and involve development of the protective chorion as well as incorporation of hepatic egg yolk proteins into the ooplasm. The penultimate development before ovulation involves the final maturation of the oocyte (FOM) when yolk proteins coalesce and the nucleus migrates to the micropyle and its membrane disintegrates. The events before FOM are coupled with a great increase in oocyte diameter and are primarily directed by the sex steroid 17β-oestradiol (E_2) (Nagahama, 1994). This hormone is usually produced from the bioconversion of testosterone (T) in the granulosa layer of the steroidogenic follicle cells surrounding the oocytes (Nagahama, 1994). Final oocyte maturation tends to be associated with a decrease in E_2 and is under the influence of progestins called maturation-inducing steroids (MIT). Although these MIT differ between species, the most common is 17α, 20β-dihydroxy-4-pregnen-3-one (17, 20βP; Patiño & Sullivan, 2002). Therefore, a detailed overview of the female reproductive cycle of a species may be determined through the seasonal analysis of these histological changes and the plasma borne steroids, which drives them.

In this study, the reproductive cycle of female *L. bergylta* caught from a wild population in western Norway was characterized. Histological data, gonad indices and plasma sex steroid values collected over two complete reproductive cycles were used to delineate ovarian recrudescence, final oocyte maturation and putative spawning. From this, the first data linking circulating steroid levels with defined stages of oogenesis in this temperate wrasse are provided.

MATERIALS AND METHODS

Gillnets and traps were used to capture 125 female *L. bergylta* from 5 to 20 m depth in western Norway (60° $06'$ N; 5° $10'$ E) between February 2005 and February 2007. Fish were obtained monthly and stored for up to 2 days in a sea cage before sampling. The fish were then rapidly removed from the storage cage by a dip-net, killed with a blow to the head and measured for total length (L_T) and body mass (M_B) to the nearest cm and g. Blood (2 ml) was then immediately obtained from the duct of Cuvier using cold heparinized syringes, stored on ice until centrifugation (12 100 *g*, 3 min; Eppendorf mini-spin; www.eppendorf.com) after which the plasma was frozen on dry ice for storage at -80° C until further analysis. Ovaries (M_G) and liver (M_L) were excised and weighed to the nearest 0·01 g for calculation of gonado-somatic (I_G) and hepato-somatic (I_H) indices according to $I_G = 100$ M_G $(M_B - M_G)^{-1}$ and $I_H = 100$ M_L $(M_B - M_L)^{-1}$.

HISTOLOGY

An introductory analysis of anterior, mid and posterior portions from both ovarian lobes indicated that oocyte development was uniform throughout the gonad. Future tissue samples were, therefore, excised from the anterior region of the ovary, fixed in neutral phosphate-buffered formalin and 3 μm paraffin sections were cut and stained with haematoxylin, eosin and saffron, using conventional protocols. Ovaries were classified according to the developmental stage of the leading cohort oocytes (West, 1990), using a light microscope with a mounted digital camera. Images were processed with Adobe Photoshop 7.0. The diameter of leading cohort oocytes that were sectioned through the nucleus was measured digitally (Image J, National Institute of Health; http://rsbweb.nih.gov/ij). The number of oocytes (*n*) required to accurately represent the diameter for each stage was determined after modelling the running normalized (moving) mean of oocyte diameter as a function of *n* from 1 to 30 (Howard & Reed, 1998; Kjesbu *et al.*, 1998) showing quickly stabilized values. Consequently, five oocytes were measured per stage reflecting an accuracy of ±2%.

STEROID ANALYSES

Steroids were extracted from the plasma samples using ether:heptane (4:1) solvents as described by Hyllner *et al.* (1994) and re-dissolved to a relevant concentration in $0·5 - 1$ ml of enzyme immunoassay buffer (EIA) (0·1 M KHPO$_4$, pH 7·4, 0·4 M NaCl, 1 mM ethylene diaminetetra acetic acid, EDTA) buffer solution (60° C, 10 min) and stored at -20° C until analysed. Circulating plasma levels of T and E$_2$ were measured by enzyme-linked immunosorbent assay (ELISA) based on the methods described by Pradelles *et al.* (1985), Maclouf *et al.* (1987) and Cuisset *et al.* (1994). Antisera, acetylcholine esterase-labelled tracers and microplates precoated with monoclonal mouse antirabbit IgG were supplied by Cayman Chemicals (www.caymanchem.com). Standard steroids were purchased from Sigma (Sigma reference standards; www.sigma-aldrich.com). Logit-log binding curves of serial dilutions of standards and plasma samples were parallel showing that the plasma extracted samples were suited to the assay conditions. The ED80 and ED20 were 0·004 and 0·08 ng ml^{-1} for T and 0·006 and 0·6 ng ml^{-1} for E$_2$. The detection limits of the assays were 0·008 and 0·015 ng ml^{-1} for T and E$_2$, respectively. Extraction efficiencies were 95% for T and 94% for E$_2$. These were determined by spiking replicate plasma samples with tritiated steroid and confirming the quantity of steroid recovered. The interassay variation was 7·7% (T) and 7·6% (E$_2$), while the intra-assay variation was 7·0% (T) and 6·8% (E$_2$). Antibody cross-reactivity is described by the supplier.

STATISTICS

Data were analysed using GraphPad Prism 5.0 (GraphPad Software Inc.; http://www.graphpad.com) and presented as mean ± S.E. Differences between groups were analysed using one-way ANOVA and Bonferroni's multiple comparison test for *post hoc* testing ($P < 0.05$). Linear regression was used to test relationships between reproductive variables, while Pearson

product-moment correlation was used to determine associations between abiotic and biotic variables. Assumptions of parametric testing were checked using Bartlett's test for equal variance and Kolmogorov–Smirnov's test for normality. In situations where data varied from these assumptions, In or inverse transformations were performed.

RESULTS

The ovaries of *L. bergylta* are arranged as a paired lobular structure, above the gut in the body cavity. The ovary is of the cystovarian type with oogonial germ cells arranged in nests inside the epithelial margin of the ovarian lamellae. During the course of the reproductive cycle, the ovaries varied in shape from a thin clear thread to a distended grey-coloured sac surrounded by a thin semi-transparent membrane (tunica albuginea). Ovarian development was classified on a six-stage scale (Table I) according to the histology of the leading cohort oocytes (Figs 1 and 2).

RELATIONSHIPS BETWEEN SEX STEROIDS, GONADO-SOMATIC INDEX AND OOCYTE DIAMETER

Linear regression showed that both plasma T ($r^2 = 0.3589$, d.f. $= 122$, $P < 0.001$) and plasma E_2 ($r^2 = 0.4746$, d.f. $= 122$, $P < 0.001$) concentrations were positively related to leading oocyte diameters [Fig. 3(a), (b)]. Low concentrations of both steroids, however, could be found in fish with different sized oocytes, indicating that plasma sex steroid concentration and oocyte size were poor predictors of each other. Linear regression analysis also indicated that I_G was dependent on oocyte

TABLE I. Stages of ovarian maturity in *Labrus bergylta*, according to histological condition and leading oocyte diameter

| Stage of maturity	Histological condition	Oocyte diameter (μm)	*n*
I Immature, recovering	Previtellogenic oocytes predominate, postovulatory follicles not present	106–174	25
II Early maturing	Leading cohort oocytes contain cortical alveoli	219–265	10
III Early yolk	Leading cohort oocytes contain small vitellogenic yolk globules and the inner zona radiata is generally <6 μm thick	307–444	23
IV Late yolk	Leading cohort oocytes contain many large yolk globules, the inner zona radiata is >6 μm thick	462–658	38
V Mature	Leading cohort oocytes contain hydrated oocytes with coalescent yolk and the germinal vesicle has started to migrate towards the animal pole	651–720	5
VI Resorbing	Atretic oocytes evident	99–314	23
		Total	124

n, number of individuals
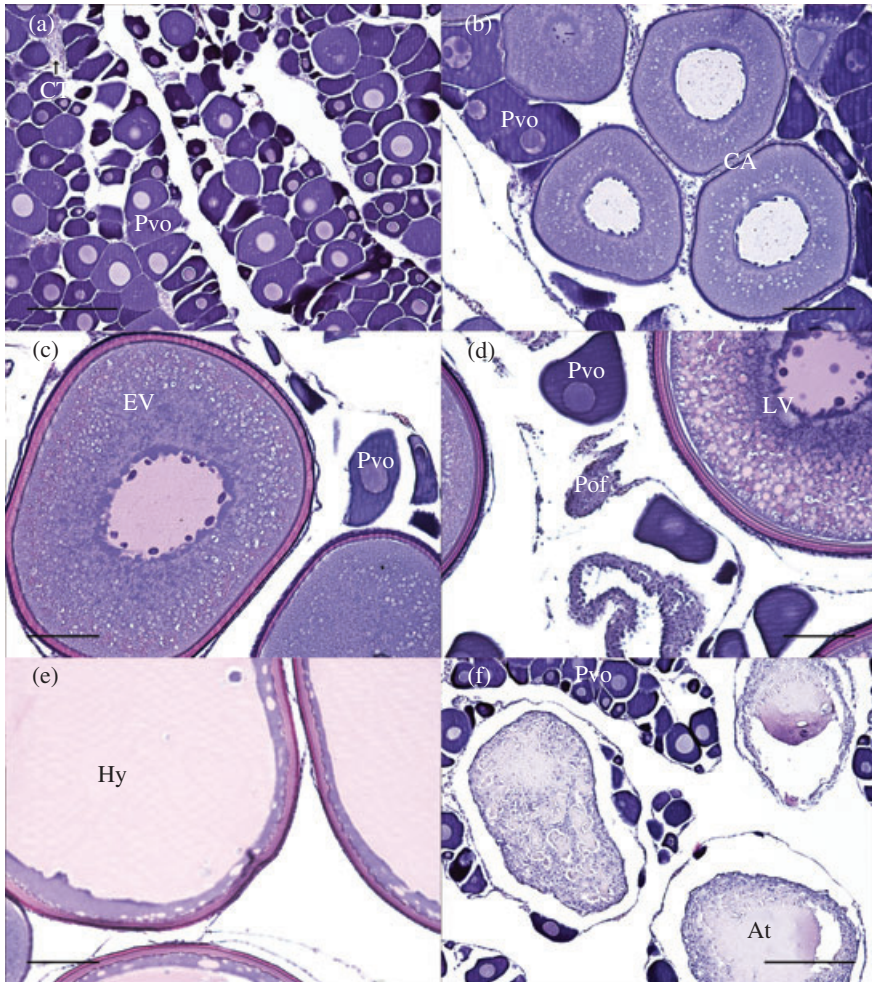
FIG. 1. Stages of ovarian development in *Labrus bergylta*: (a) stage I, recovering, (b) stage II, early maturing, (c) stage III, early yolk, (d) stage IV, late yolk, (e) stage V, mature and (f) stage VI, resorbing (see Table I). Scale bars = 100 μm (a), (b), (c), (d) and (e) and 250 μm (a) and (f). At, atretic oocyte; CA, cortical alveolus oocyte; CT, connective tissue; EV, early vitellogenic oocyte; Hy, mature hydrated oocyte; LV, late-vitellogenic oocyte; Pof, postovulatory follicles; Pvo, previtellogenic oocyte.

diameter [$r^2 = 0.7850$, d.f. $= 121$, $P < 0.001$, Fig. 3(c)]. The maximum I_G, T and E_2 levels recorded were 15·6, 0·87 and 3·83 ng ml^{-1}, respectively.

SEX STEROIDS, GONADO-SOMATIC AND HEPATO-SOMATIC INDICES IN RELATION TO OVARIAN MATURITY

Stages of ovarian maturity differed significantly (one-way ANOVA, $n = 125–127$, $P < 0.001$) in I_H, I_G and concentrations of E_2 and T (Fig. 4). Both T and E_2 concentrations reflected a similar trend in relation to ovarian stage. There were no
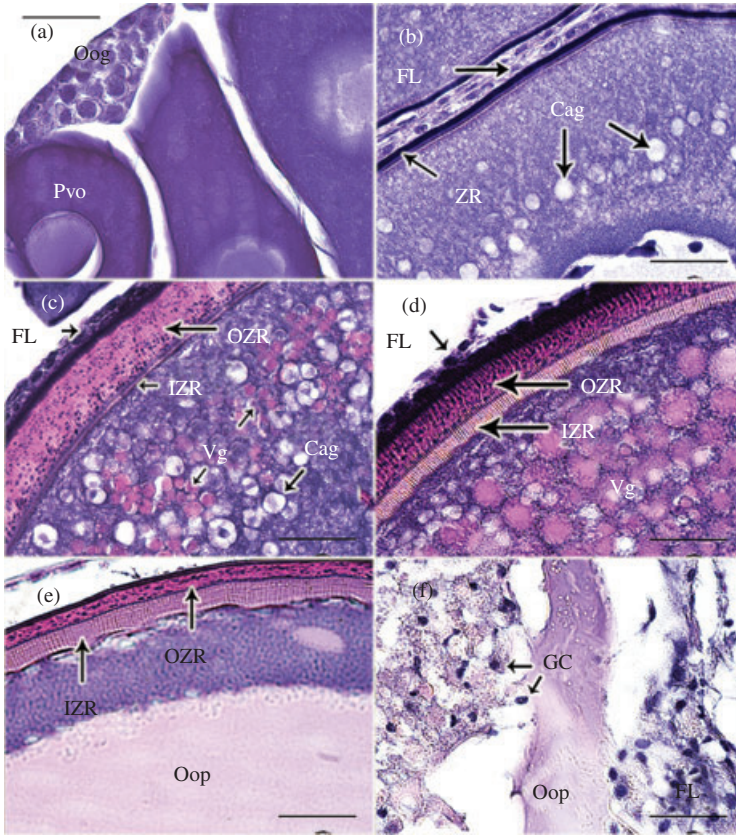
FIG. 2. Enlargement of ovarian developmental stages in *Labrus bergylta*: (a) stage I, recovering, (b) stage II, early maturing, (c) stage III, early yolk, (d) stage IV, late yolk, (e) stage V, mature and (f) stage VI, resorbing (see Table I). Scale bars = 25 μm (a), (b), (c), (d), (e) and (f). Cag, cortical alveoli granules; FL, follicular layer; GC, granulosa cells; IZR, inner zona radiata; Oog, oogonia; Oop, ooplasm; OZR, outer zona radiata; Pvo, previtellogenic oocyte; Vg, vitellogenin globules; ZR, zona radiata.

significant differences between stages I (immature) and II (early maturing) and minimum concentrations of 0.04 ± 0.01 ng ml^{-1} for T and 0.06 ± 0.01 ng ml^{-1} for E_2 were recorded. Steroid levels increased in fish with stage III (early vitellogenic) ovaries and were maximal in stage IV (late vitellogenic) (0.24 ± 0.03 ng ml^{-1} and 1.08 ± 0.14 ng ml^{-1} for T and E_2, respectively). Concentrations of T and E_2 decreased in stage V (mature), although these were not significantly different from stage IV values. There was a further decrease of T (0.05 ± 0.01 ng ml^{-1}) and E_2 (0.06 ± 0.02 ng ml^{-1}) in fish with stage VI (resorbing) ovaries to near minimal levels.

The I_G increased significantly from a minimum value of 0.86 ± 0.05 in fish with stage I ovaries to a maximum value of 11.66 ± 1.23 in fish with stage V gonads. There was no significant difference, however, between fish with stages IV (6.92 ± 0.46) and V ovaries. Fish with stage VI ovaries had near-minimal I_G (1.16 ± 0.05). The I_H did not vary greatly although differences were evident among the six stages
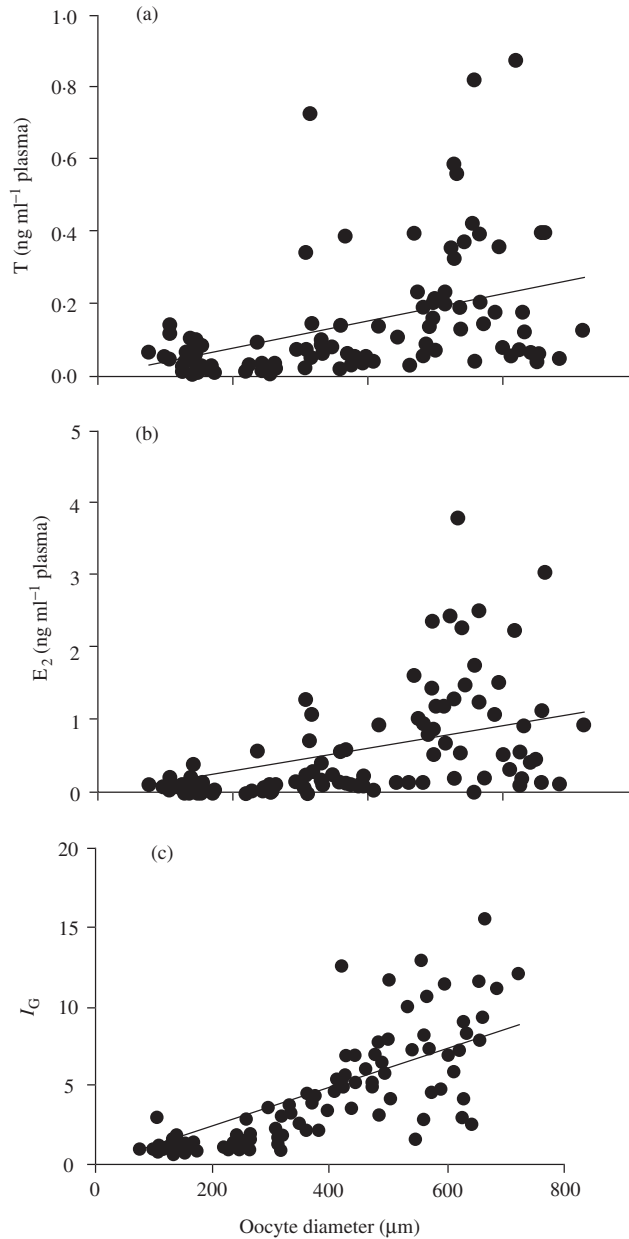
FIG. 3. Leading oocyte diameter in relation to (a) levels of plasma testosterone (T) and (b) plasma 17β-oestradiol (E$_2$) and to (c) the gonado-somatic index (I_G) in female *Labrus bergylta*. The curves were fitted by: (a) $y = 0.0004x$, (b) $y = 0.0016x$ and (c) $y = 0.1220x$.

of ovarian development. Fish with stages I and II ovaries had a similar I_H of c. 1.5. This increased to 1.86 ± 0.08 in stage III and attained maximal value of c. 2.2 in fish with stages IV and V ovaries. There was a slight decrease of I_H in fish with stage VI ovaries to 2.00 ± 0.13.
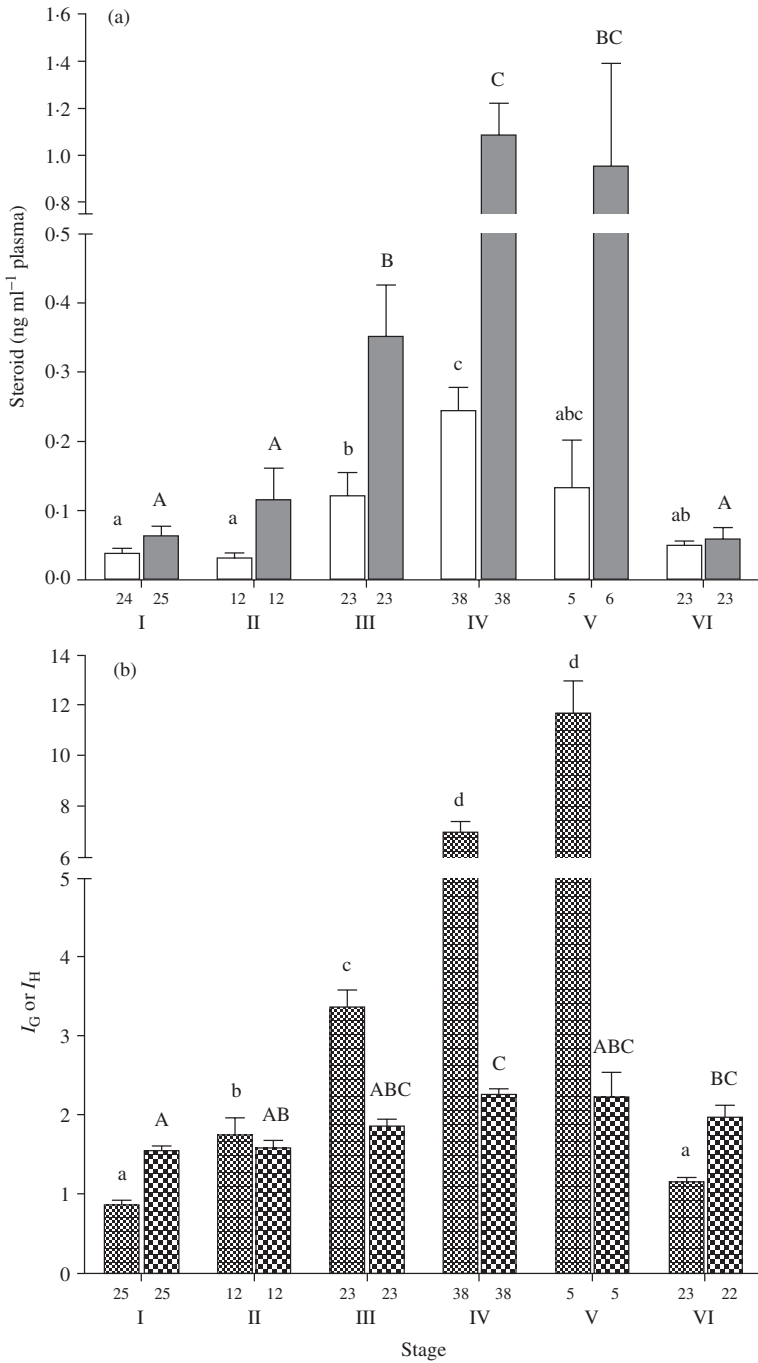
FIG. 4. Mean ± S.E. (a) plasma testosterone (T, □) and 17β-oestradiol (E₂, ■) and (b) gonado-somatic index (I_G, ▦) and hepato-somatic index (I_H, ▦) between different stages (see Table I) of ovarian development in *Labrus bergylta*. Numbers beneath bars indicate sample sizes. Letters indicate significant differences ($P < 0.05$) between stages of each variable (lower cases = T or I_G, upper cases = E₂ or I_H) as determined by one-way ANOVA and Bonferroni's multiple comparisons test.
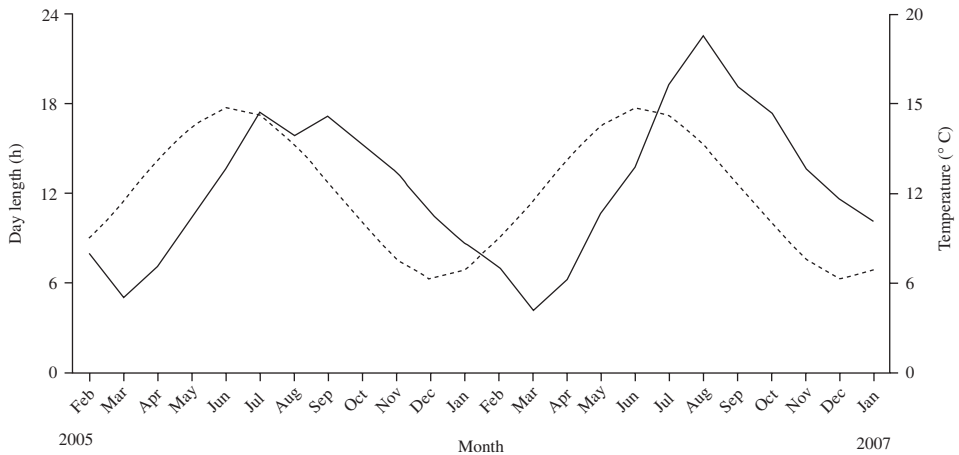
F_{IG}. 5. Mean monthly values of seawater temperature (——) and day length (······) between February 2005 and January 2007 at $60° 06' 0'' $ N; $5° 10' 0'' $ E.

MEAN MONTHLY VALUES OF PLASMA SEX STEROIDS AND GONADO-SOMATIC INDEX IN RELATION TO ENVIRONMENTAL FACTORS AND OVARIAN MATURITY

Values of sex steroids and I_G showed cyclic changes during the 2 years of study. Sea temperature also followed an annual cycle in a similar but slightly delayed trend to that observed in day length. The mean minimum and maximum seawater temperatures for this period were $3·5 ± 0·1°$ C and $18·8 ± 0·1°$ C, respectively (Fig. 5). Negative correlations existed between sea temperature and plasma T ($r = -0·4867$, d.f. $= 22$, $P < 0·05$) and E_2 ($r = -0·5921$, d.f. $= 22$, $P < 0·01$) levels and I_G ($r = -0·6791$, d.f. $= 22$, $P < 0·01$). There were no correlations between day length and plasma T, E_2 or I_G.

Minimum values of T, E_2 and I_G were recorded as day length and seawater temperatures decreased, this was *c*. 2 months after maximal seawater temperatures and *c*. 2 months before the winter solstice (Figs 5 and 6). In contrast, these physiological variables were maximal as sea temperature and day length increased, with peak values recorded in the 2 months before the summer solstice and *c*. 2 to 4 months before sea temperature maxima. There was often an overlap of 1 or 2 months during which individual fish were recovered containing ovaries of either one or another stage of maturity. Fish with stage I ovaries ($n = 25$) were found between August and December, although a total of only four fish in this stage were found between September and December. Fish with stage II ($n = 10$) ovaries occurred between November and February, with only one fish found in this stage in February. Stage III ovaries ($n = 23$) were evident from January to April, one fish in this stage was also found in June. Stage IV ovaries ($n = 38$) existed between March and June, the majority of fish caught in May and June had ovaries in this stage. Five fish with stage V ovaries were obtained during this study, one was in April and the remaining four were found in May and June, these latter 2 months also coincided with the occurrence of postovulatory follicles (POF). Only fish with stage VI ovaries ($n = 23$) occurred in July
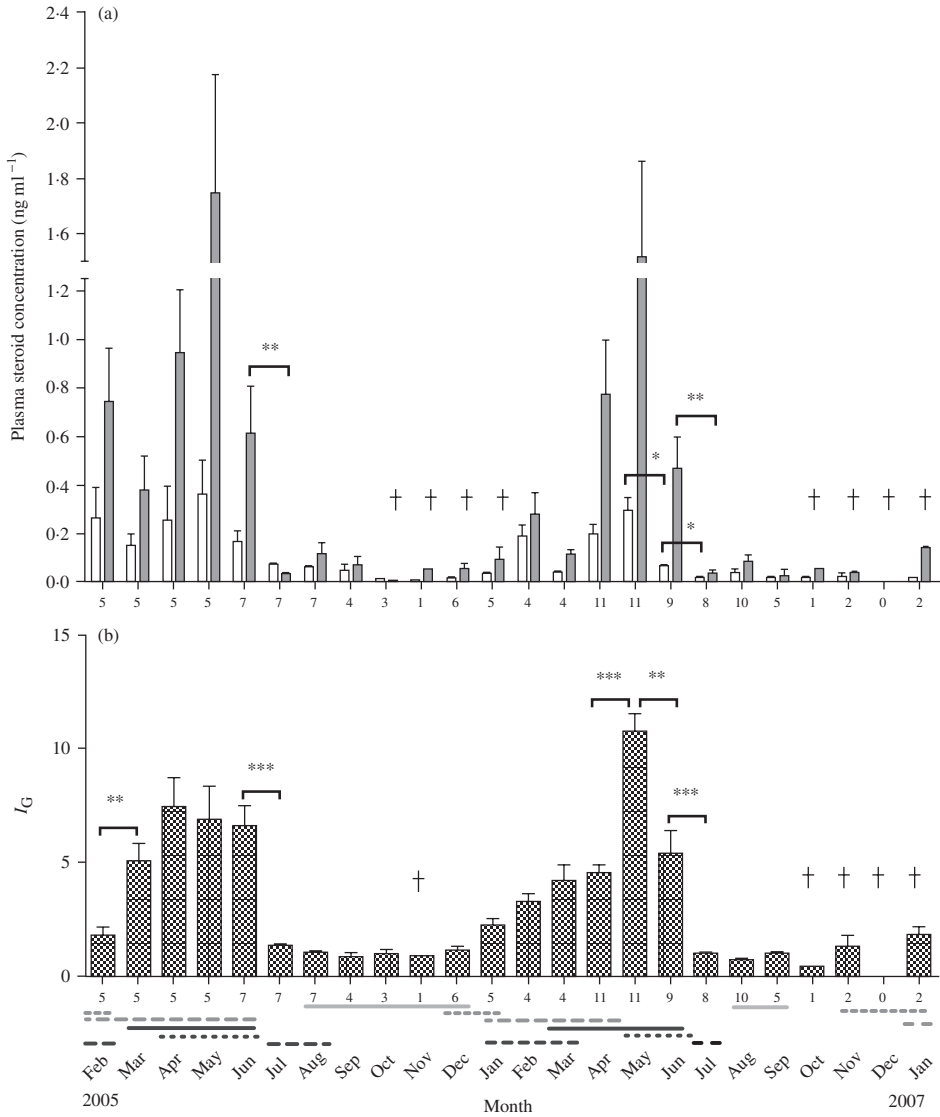
FIG. 6. Mean ± S.E. monthly values of (a) plasma testosterone (□) and 17β-oestradiol (■) and (b) gonado-somatic index (I_G) and stage of ovarian development in female *Labrus bergylta* between 2005 and 2007. ——, stage I; - - -, stage II; – –, stage III; ——, stage IV; •••, stage V; - - -, stage VI (see Table I). Numbers beneath the bars indicate sample size while cross symbols denote months dropped from analysis due to insufficient samples size. The level of significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) between consecutive months as determined by one-way ANOVA and Bonferroni's multiple comparisons test is indicated.

($n = 15$), five fish of this stage were also present in August and one in January, February and March.

Values of T, E_2 and I_G varied significantly (one-way ANOVA, $n = 127$, $P < 0.001$) in relation to month of the year. Minimum T concentrations ranging from 0.01

to 0.07 ± 0.01 ng ml^{-1} occurred between July and January. Although not significantly different from previous months, a higher mean value of 0.19 ± 0.05 ng ml^{-1} was recorded in February of the second year. The mean value of T in February of the first year was even higher (0.27 ± 0.12 ng ml^{-1}). Lower values were recorded in March of both the first (0.15 ± 0.05 ng ml^{-1}) and second years (0.04 ± 0.01 ng ml^{-1}). Values in April were similar to February in the same years (0.25 ± 0.14 and 0.20 ± 0.04 ng ml^{-1} for years 1 and 2, respectively). The highest values were recorded in May of years 1 (0.36 ± 0.14 ng ml^{-1}) and 2 (0.30 ± 0.05 ng ml^{-1}). These values decreased significantly between May and June (0.07 ± 0.01 ng ml^{-1}) of the second year. The following month of June was characterized by lower T values in both years (0.17 ± 0.04 and 0.07 ± 0.01 ng ml^{-1}, respectively); however, there was only a significant decrease in the second year.

The annual profile of E_2 concentrations followed a similar trend to T although most values were not significantly different between months. Maximal E_2 values were approximately five-fold greater than T. Minimum E_2 values of 0.01 to 0.14 ± 0.01 ng ml^{-1} occurred between July and January. The E_2 concentration in the second year doubled from January (0.14 ± 0.01 ng ml^{-1}) to February (0.28 ± 0.09 ng ml^{-1}). Values of E_2 in March (0.38 ± 0.14 and 0.12 ± 0.02 ng ml^{-1}, years 1 and 2, respectively) were approximately half of those recorded in the previous February. This contributed to the formation of an annual bimodal peaked trend similar to that observed for T concentrations. Higher values were recorded in April of both years (0.95 ± 0.26 ng ml^{-1} in year 1 and 0.78 ± 0.22 ng ml^{-1} in year 2), while maximal values of E_2 were recorded in May (1.75 ± 0.43 and 1.51 ± 0.35 ng ml^{-1}, respectively). Lower E_2 values were recorded in June 0.62 ± 0.19 and 0.47 ± 0.13 ng ml^{-1}, respectively. There was a significant decrease in E_2 concentrations between June and July of both years.

Changes in I_G approximated a similar trend to that of T and E_2 over the 2 year period. There was no evidence, however, of an annual bimodal peak between February and May. In contrast, I_G values were greater in March than in February, although there was only a significant increase in the first year. Values were low between July and December (0.44 to 1.35 ± 0.11). Higher values were recorded in January of each year (2.25 ± 0.31 and 1.87 ± 0.34 for years 1 and 2, respectively). The highest I_G values were recorded between April and June in both years (6.61 ± 0.88 to 7.44 ± 1.27 in year 1 and 4.57 ± 0.35 and 10.71 ± 0.81 in year 2). Peak values, however, were recorded in April in the first year and in May in the second with lower values recorded in June (6.61 ± 0.88 in year 1 and 5.41 ± 0.97 in year 2). Peak values in May of the second year were significantly higher than both April and June of the same year. There was a significant decrease in I_G between June and July.

DISCUSSION

This study characterizes the reproductive cycle of female *L. bergylta* in Norway using plasma T and E_2 and I_G, oocyte diameter and ovarian histology, collected monthly over a 2 year period. Ovarian development of *L. bergylta* followed a clear annual cycle. Such defined reproductive cycles are typical of temperate and high latitude teleosts (Crim, 1982). Seasonal oscillations often permit only limited periods where environmental conditions are conducive to the survival of offspring. The

L. bergylta in this study appeared to enter a short quiescent period between August and October, when I_G was at minimal levels and only previtellogenic oocytes and nests of oogonia were present in the ovary (stage I). A few fish with postspawned, resorbing ovaries (stage VI) containing atretic postvitellogenic oocytes, however, were still present in August of the first year. Atresia of unspawned oocytes was complete by September. This was also when oocyte diameter was lowest. Plasma concentrations of T and E_2 were minimal in September and October, suggesting the existence of a brief quiescent period in late summer, autumn as light period and sea temperature started to decrease.

Ovarian recrudescence started in November and December with the appearance of oocytes containing cortical alveoli (stage II) and a trend of greater E_2 and I_G values. A continued trend of greater T, E_2 and I_G values occurred 2 months later in January with the appearance of early vitellogenic oocytes (stage III ovaries). Two steroid peaks were indicated in February and April with a trend for lower steroid concentrations in March. Although these apparent bimodal steroid peaks were consistent in both years, the concentrations did not differ significantly between consecutive months. Nevertheless the pattern is interesting as bimodal steroid profiles have been observed in the ovarian recrudescence of other teleosts (Bonnin, 1979; Burke *et al.*, 1984; Pankhurst & Conroy, 1987, Prat *et al.*, 1990). A significant increase in I_G during these months was only evident in the first year of sampling, however, further distorting any apparent connection between ovarian development and steroid bimodality. While the significance of bimodality may be unclear, the observed increase of I_G in the first year may be explained by E_2 stimulation of vitellogenesis (Nagahama, 1994).

Gonadal steroid production was greatest during vitellogenic stages of oocyte development. The lipophosphoglycoprotein, vitellogenin is produced in the liver under receptor-mediated stimulation of E_2 in teleosts (Menuet *et al.*, 2001). Therefore, oogenesis proceeded rapidly from February to May as E_2 concentrations increased, and by March approximately half of the fish contained stage IV ovaries with late-vitellogenic oocytes. This was reflected by significant differences in I_G among fish with stages II, III and IV ovaries. Vitellogenesis often causes a temporary increase in liver mass (Korsgaard-Emmersen & Emmersen, 1976; Bohemen *et al.*, 1981; Haux & Norberg, 1985). This is likely to explain the significant increase in I_H between fish with stages II and IV ovaries. Indeed, the increase in I_H observed in stages III and IV ovaries is supported histologically through the vitellogenin incorporation evident in the oocytes of these fish. In addition, the thickness of the inner zona radiata (IZR) increased in fish with stage IV oocytes (Table I). These choriogenins are often derived from the liver, although not exclusively in all teleosts (Begovac & Wallace, 1989; Chang *et al.*, 1996; Hyllner *et al.*, 2001), under the influence of E_2 and may have, in part, contributed to the increase in I_H in these stages. Therefore, much of the observed increase in I_G and I_H is associated with oocyte development resulting from increased sex steroid production.

The plasma steroid levels decreased as vitellogenesis ended. Although not significant, the mean plasma concentrations of T and E_2 were slightly less in fish containing stage V ovaries with mature, hyaline oocytes. Aromatizable androgens and E_2 have been shown to have a negative feedback on the pituitary release of follicle-stimulating hormone (FSH) and a potentiating effect on luteinizing hormone (LH) release, in many teleosts (Yaron *et al.*, 2003). This results in a shift towards the follicular production of progestogens that stimulate final oocyte maturation and may explain the

decreased E_2 concentrations observed in fish with stage V ovaries. The patterns of FSH and LH release can be complicated depending on the spawning strategy of the fish (Yaron *et al.*, 2003). The first incidence of a fish with stage V ovaries was in late April, indicating that spawning was imminent. Maximal concentrations of T and E_2, however, were not until May, after which, there were marked decreases in T, E_2 and I_G in June. This indicates that peak spawning occurred during these 2 months. Rapid decreases in E_2 and I_G from peak values are typical patterns of gonad maturation and spawning in other temperate marine teleosts (Shimizu, 1997; Dahle *et al.*, 2003; Clark *et al.*, 2005; Garcia-López *et al.*, 2006). The ovaries of fish in May and June contained an abundance of POF and were in either stage IV or V, indicating that the spawning season started before May. Spawning seems to be complete by July as indicated by ovaries in a postspawned state with atretic mature oocytes (stage VI). Moreover, this notion was also supported by a significant decrease in T (year 2), E_2 and I_G, as well as the deflated appearance of the oocytes that had been invaded by phagocytotic granulosa cells (Lang, 1981). Previous work in British waters classifies *L. bergylta* as a group-synchronous single spawner (Dipper & Pullin, 1979). The coincidence of POF together with oocytes in several developmental stages and the existence of a large pool of previtellogenic oocytes in the present study indicates that *L. bergylta* should be classified as a group-synchronous multiple spawner. This was further supported by the observation that individual fish can spawn at least three times in the same breeding season (unpubl. obs.).

Seasonal shifts of environmental variables are likely to modulate the reproductive cycle of *L. bergylta*. Although there was no clear association between changes in day length and the production of T, E_2 or I_G, it remains likely that shifts in photoperiod are involved in the timing of reproduction in this species. Photoperiod is a primary cue affecting the timing of reproduction in most teleosts (Hoover, 1937; Girin & Devauchelle, 1978; Whitehead *et al.*, 1978; Smith *et al.*, 1991; Taranger & Hansen, 1993; Watanabe *et al.*, 1998; Morehead *et al.*, 2000). In addition, changes in day length are a dominant environmental phenomenon at the latitude of this study. Correlations between sea temperature and T, E_2 and I_G indicate that temperature has a significant effect on gonadal processes and reproduction. Temperature has been suggested to fulfil a permissive role in teleost reproduction (Taranger *et al.*, 2010). A decrease in temperature appears to induce the reproductive cycle in percids and moronids (Dabrowski *et al.*, 1996; Prat *et al.*, 1999; Migaud *et al.*, 2002; Clark *et al.*, 2005). This may also be consistent with the inverse correlations observed between sea temperature and steroid levels as well as I_G, in *L. bergylta*.The decrease in T, E_2 and I_G in June, as well as the minimal levels of these variables associated with postspawning in July, contrasts with the rapidly warming sea temperatures at this time of year and could also help explain the inverse association between these biotic and abiotic factors. Although temperature may influence enzymatic processes in the gonads, the data from this study do not support greater speculation. Further research into the relationship between the photothermal cues and the reproductive cycle of *L. bergylta* will have important implications for controlling reproduction in this species.

Plasma sex steroid concentrations in *L. bergylta* were found to be relatively low (<5 ng ml^{-1}) like several other marine species such as the saddleback wrasse *Thalassoma duperrey* (Quoy & Gaimard) (Nakamura *et al.*, 1989), the blackeye goby *Coryphopterus nicholsii* (Bean) (Kroon & Liley, 2000), honeycomb grouper *Epinephelus merra* Bloch (Bhandari *et al.*, 2003) and red porgy *Pagrus pagrus* L.

(Kokokiris *et al.*, 2006), which have sex steroid levels between 1 and 10 ng ml^{-1}, By contrast, other fish species such as salmonids may have concentrations of >50 ng ml^{-1} (Taranger *et al.*, 1998). Low sex steroid production may be typical of fishes that spawn multiple times within a spawning season. Pankhurst & Carragher (1991) suggest that fishes with low steroid levels tend to follow a semi-lunar spawning cycle while group-synchronous spawners such as plaice *Pleuronectes platessa* L. and winter flounder *Pseudopleuronectes americanus* (Walbaum) often have higher sex steroid levels. It would seem that *L. bergylta* also fits this trend as an apparent group-synchronous multiple spawner and low steroid levels. This would be better supported through captive manipulation of fish and the measurement of sex steroids across a finer time scale as E$_2$ concentrations can fluctuate between the spawning of egg batches (Zohar *et al.*, 1998; Methven *et al.*, 1992; Kjesbu *et al.*, 1996). Further attention also needs to be applied to identifying final maturation-inducing steroids in these fishes. For example, the Japanese kyusen *Halichoeres poecilopterus* (Richardson) and bambooleaf *Pseudolabrus japonicus* (Houttuyn) wrasses both produce two progestins, 17, 20β-dihydroxy-4-pregnen-3-one (17, 20βP) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) responsible for inducing germinal vessicle breakdown in oocytes (Matsuyama *et al.*, 1998*a*, 2002). Quantification studies of these progestins in combination with analysis of ovarian histology have determined that these fishes follow a diurnal spawning cycle with peak spawning occurring between 0600 and 0900 hours or presumably shortly after dawn (Matsuyama *et al.*, 1998*b*, 2002). Therefore, identification and measurement of MIS progestins would provide valuable information regarding the frequency and timing of ovulation in *L. bergylta*.

In summary, female *L. bergylta* at 60° N experiencing water temperatures between 4 and 19° C and a day length oscillation of 6 to 18 h follow an annual reproductive cycle that is characterized by increasing values of T, E$_2$ and I_G and culminates in spawning in April to June. Early ovarian recrudescence begins in late autumn to early winter when day length is near minimal and water temperature is decreasing. Oocytes rapidly progress into vitellogenesis by late winter. Spawning appears to take place in late spring to early summer, when both day length and water temperature are increasing, and is complete shortly after the summer solstice as sea temperature continues to increase. Postspawned fish undergo ovarian atresia between July and August before possibly entering a short resting period.

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