EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON GONADAL DEVELOPMENT OF LUMPFISH (*CYCLOPTERUS LUMPUS*).

Currently, lumpfish are used in salmon sea cages to control the lice but off-season production of lumpfish juveniles is not possible due to lack of knowledge of reproductive biology and physiology of farmed lumpfish. Manipulations of photoperiod and temperature in temperate farmed fishes affect sexual maturation and spawning. In the CycloBreed project, we studied and described the effects of photoperiod and temperature on gonadal development and sex steroid profiles. We have also developed a non-invasive sex determination method of lumpfish using ultrasound.

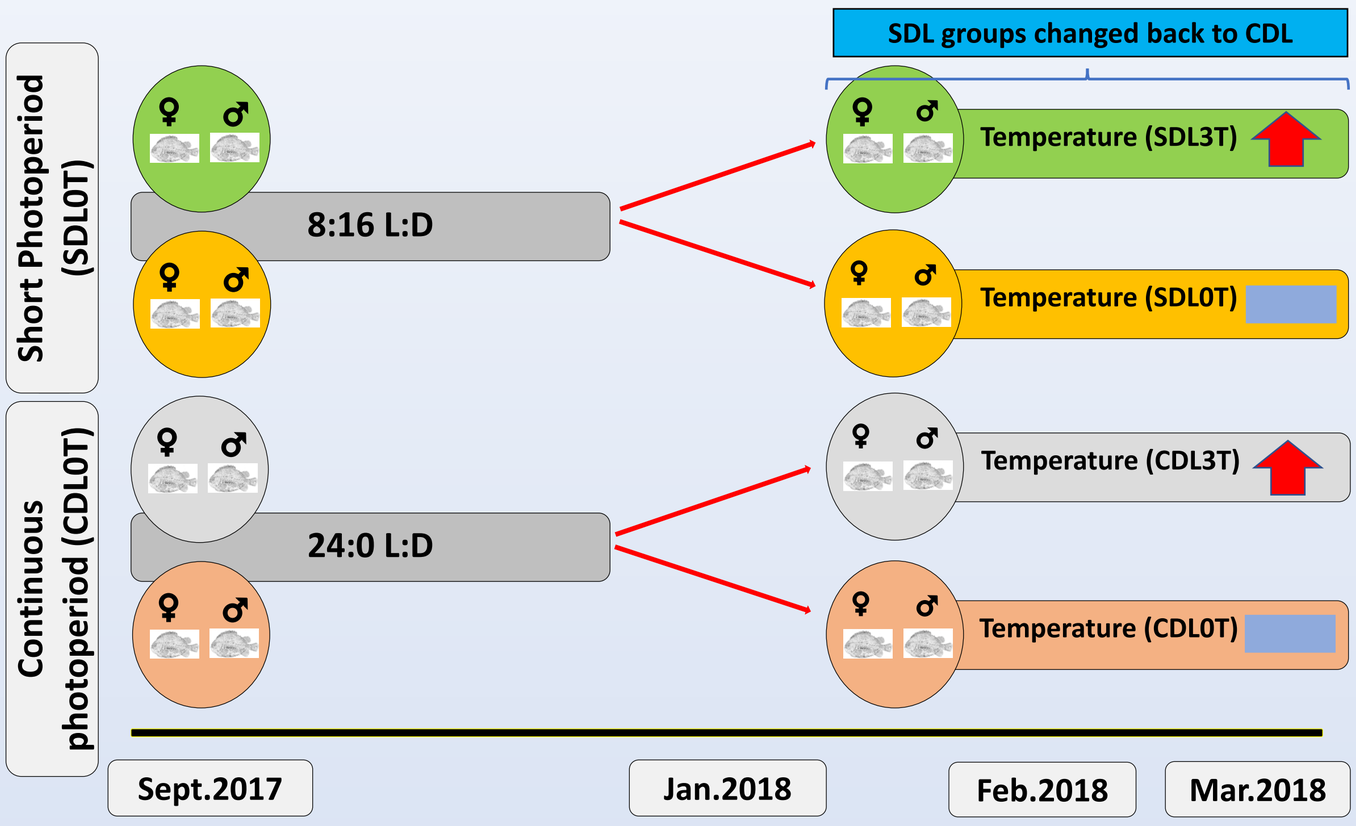

Fig. 1. Experimental Setup.

Lumpfish used for this experiment were reared under continuous light from hatching onwards. After 18 months, fish were weighing an average of 697.9g (± 364.1g), and with average length of 25 cm (±3.8cm). Four tanks (1500 l each) were stocked with 75 fish tank^{-1}, and a M:F sex ratio of 1.08. The tanks were grouped into two photoperiod groups and all tanks at ambient temperature, as shown in Fig.1. Four months later, the temperature in one tank for each photoperiod was increased by 3°C (CDL3T and SDL3T), and photoperiod in both SDL tanks changed back to CDL. There were four sampling points, in which, body and gonad weights were measured. Blood plasma for the analysis of changes in sex steroid levels and gonad sections for histological gonad development were also acquired. Based on oocyte types, ovaries were categorized into pre-vitellogenesis, early vitellogenesis, late vitellogenesis, final maturation and ovulation. Testes were categorized based on the cell types into spermatogonia, spermatocytes, spermatids and spermatozoa.

Results

Fig. 2: Variation in gonadal development in males (bottom) and females (top).

A large variation in gonadal development was observed in males and females of same size (gender-wise comparison) within the same treatments. Ovaries of sexually matured females were heterogenous, with unovulated oocytes and ovulated oocytes situated dorsally and ventrally (green arrow on the top right in Fig. 2), respectively in the ovary. The heterogenous nature of the ovaries can be linked with the multiple batch spawning behaviour of lumpfish..

Mean weight (g ±SD)	n	Correct total (%)	Correct males (%)	Correct females (%)
48 ± 14	30	94	92	92
65 ± 10	20	87	100	71
72 ± 16	35	100	100	100
157 ± 42	24	85	85	77
205 ± 56	39	69	72	67
267 ± 72	34	91	89	94
417 ± 119	32	97	94	100
610 ± 206	34	91	82	100

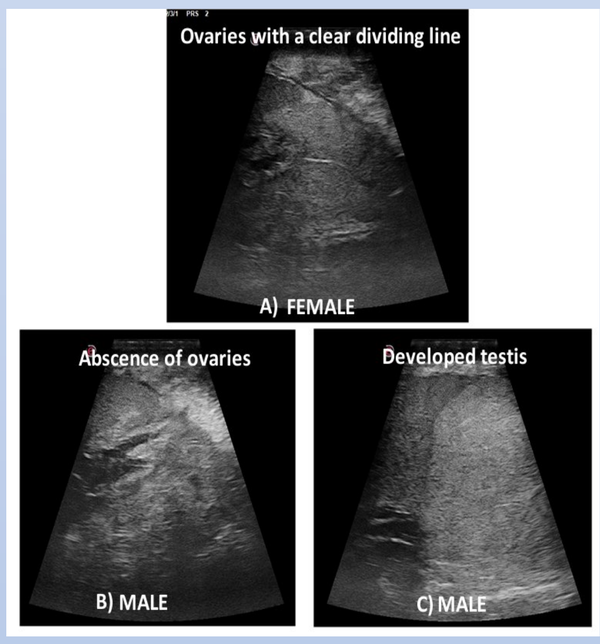

Table showing the degree of gender identification of males and females of lumpfish at different size. Figure 4: Ultrasound images of male and female lumpfish.

In juvenile, commercial sized lumpfish (48 ± 14 to 72 ± 16 g), sex was correctly determined by ultrasound examination in 87 to 100 % of lumpfish, with highest accuracy in lumpfish of 72 ± 16 g. In larger lumpfish (192 ± 53 g to 742 ± 310 g), sex determination accuracy varied between 69 to 100 % for both sexes. In females, an accuracy of 100 % was achieved at a smaller size (392 ± 141 g) compared to males (742 ± 310 g). In all larger lumpfish (up to 1438 ± 872 g), sex was correctly determined and was related to an increase in GSI. Male coloration became visible in males at 392 ± 141 g and increased from 44 % to 96 % during the last examination at 1438 ±872 g. The sex of immature males lacking coloration was determined with 100 % accuracy using ultrasound in 742 ± 310 g and larger males. Ultrasound seems to be will suited as a non-invasive method for sex determination in lumpfish. We will further compare our observations to external morphological measurements of lumpfish and test how the handling during ultrasound examination influences survival.

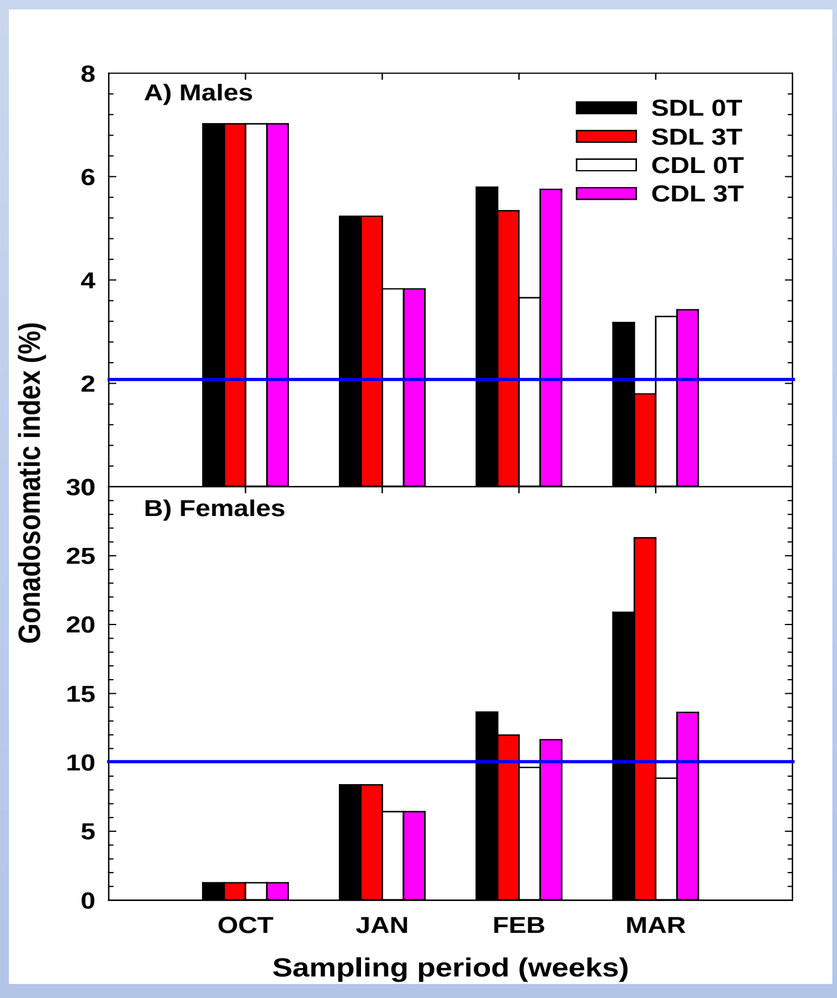

Fig. 3: GSI of Male and Females

Males did not respond to the photoperiod or temperature. There were mature males throughout the study. No significant differences existed among different treatments in GSI of males. Females responded well to the photoperiod and also to some extend to temperature. More females were matured in the short-day length treatment compared to continuous light treatment. Similarly, treatments received 3°C increase showed higher GSI.

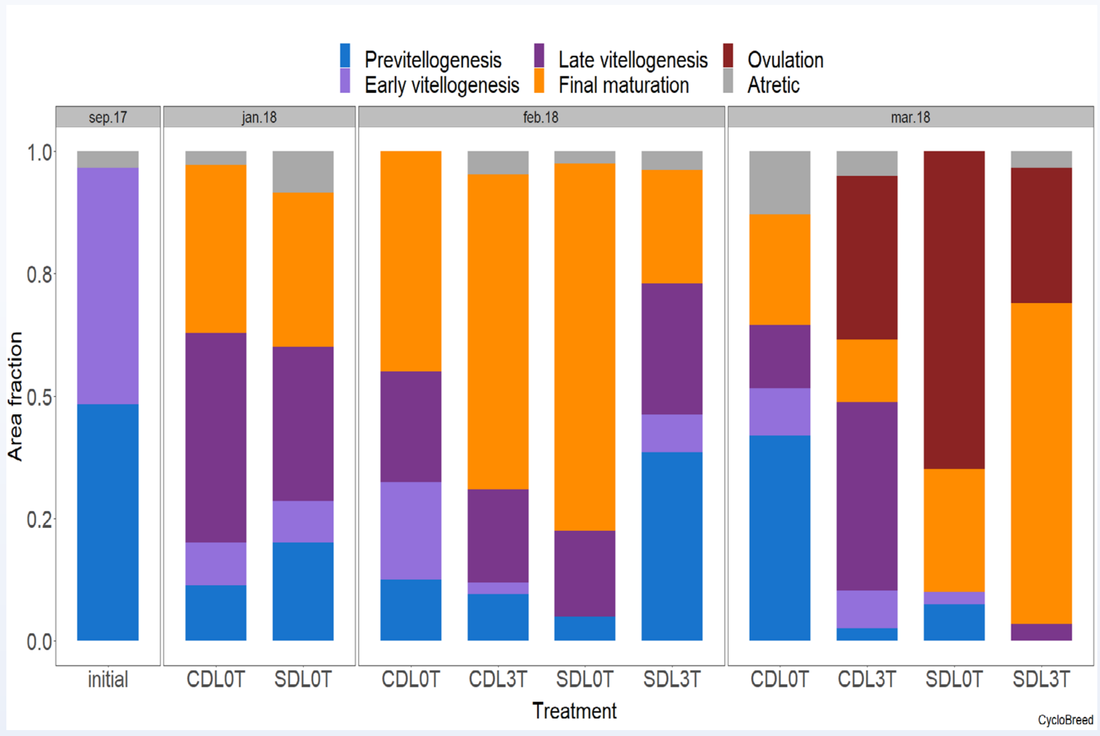

Fig. 5. Temporal changes in the area fraction of different ovarian developmental stages of lumpfish females in different treatments.

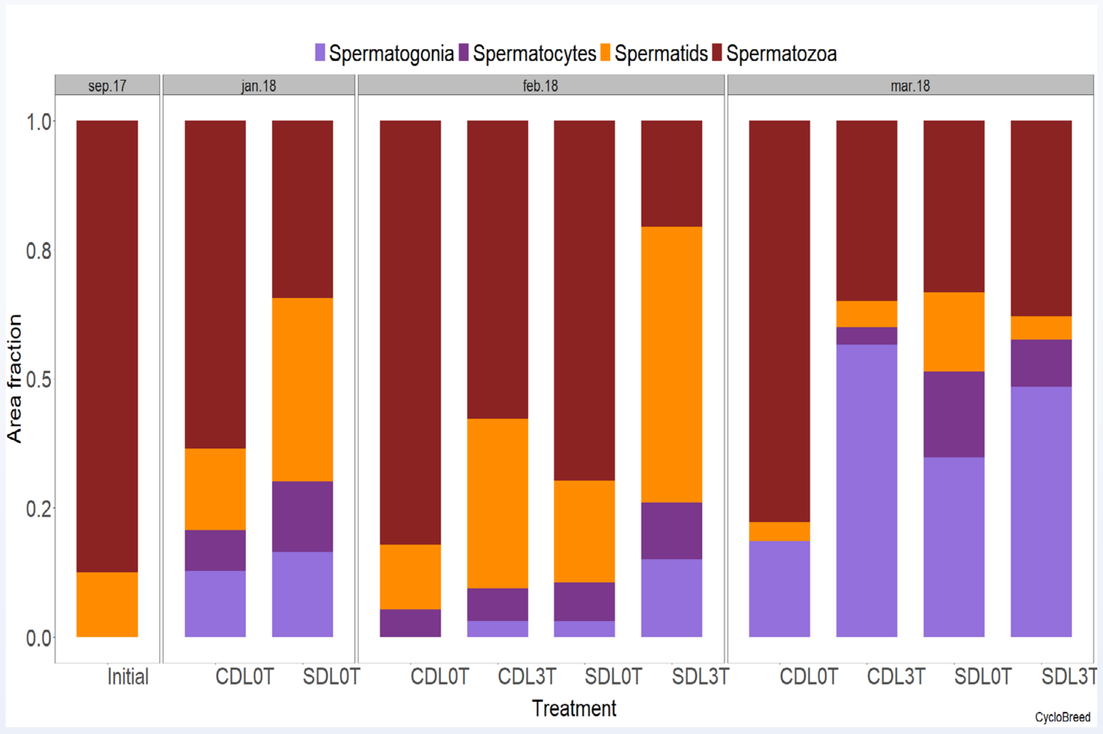

Fig. 6. Temporal changes in the area fraction of different testes developmental stages of lumpfish males in different treatments.

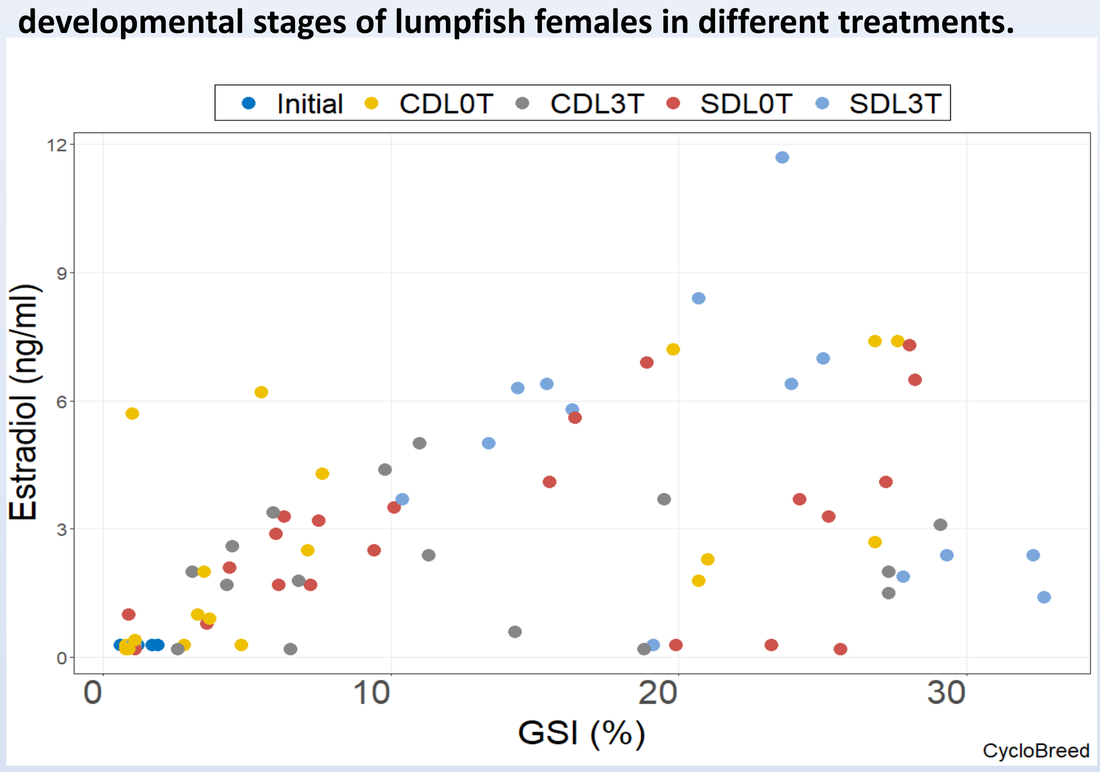

Fig. 7. Correlation between GSI and estradiol 17- β levels of lumpfish females in different treatments.

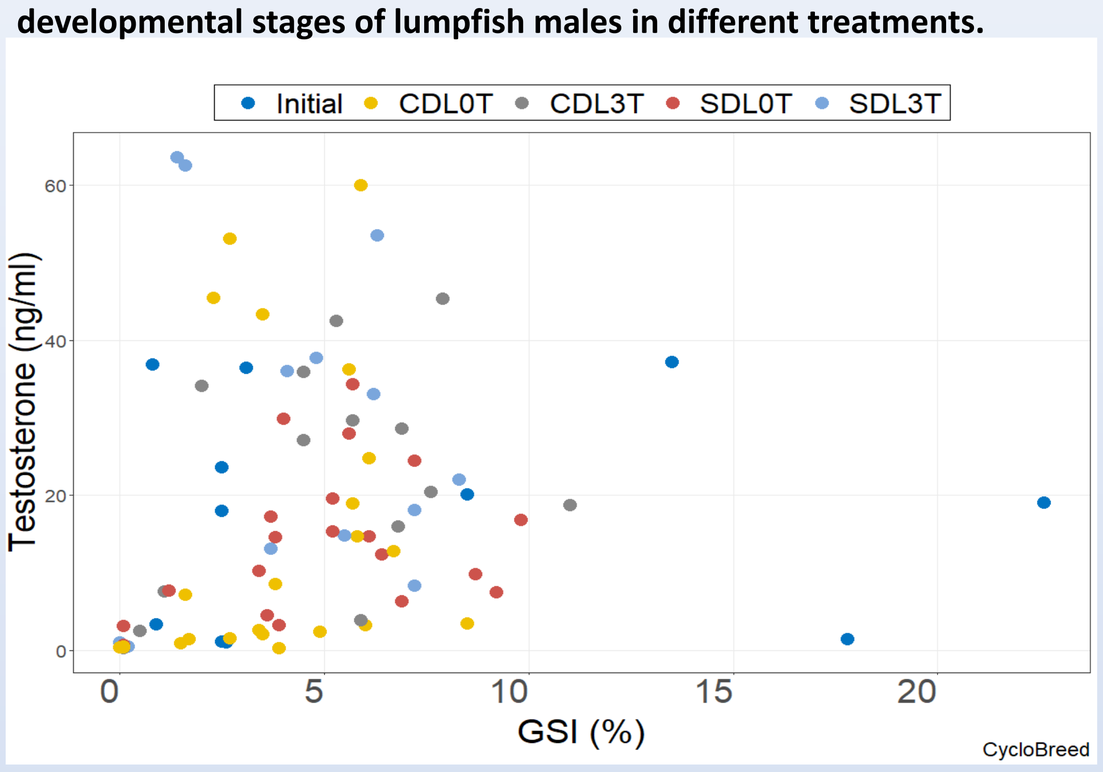

Fig. 8. Correlation between GSI and testosterone levels of lumpfish males in different treatments.

In female lumpfish, only pre-vitellogenic and early vitellogenic ovaries were observed at the start in September 2017. In January 2018, CDL0T group had the most developed ovaries at late vitellogenesis and final maturation. In February 2018, the SDL0T group had most ovaries in final maturation. In March 2018, the largest proportion of ovaries in ovulation was observed in the SDL0T group. Estradiol 17- β (E2) and Testosterone (T; not shown here) levels in females increased with time and were higher in the SDL0T groups than CDL0T groups, even higher at high temperature. Testes showed a high variation in development, and mature males were observed from the start throughout the experiment. T and 11-Ketotestosterone (11-KT; not shown here) levels in males were highest in February 2018 and decreased towards March 2018.

Conclusions and recommendations

In our study, different photoperiod did not affect the growth of lumpfish and is different the from study by Imsland et al. (2018) in which continuous photoperiod promoted the somatic growth in lumpfish. Our study showed that gender identification can be successfully done using ultrasound and in most cases, above 90% correctly. Ovary development was higher in the short photoperiod groups, similar to previous observations that continuous photoperiods may delay or inhibit gonadal development (Davie et al., 2007). The low levels female sex steroids in the continuous photoperiods agree with low testosterone and estradiol 17-β levels related with exposure of haddock to continuous photoperiod (Davie et al., 2007). The role of temperature in timing of maturation and spawning observed in wolffish (Tveiten and Johnsen, 1999) tempts to suggest that, lumpfish ovary maturation is affected by temperature, due to the observed effects of increasing temperature. Spawning differences among the photoperiod groups agree with Imsland et al. (2019), who found that, lumpfish spawn sparsely under continuous photoperiod, but distinctly under compressed photoperiods. This is the first study to describe gonad development in farmed lumpfish histologically. It is further demonstrated that, photoperiod and temperature manipulations can be applied to control sexual maturation and spawning of broodstock, for better broodstock management and ensured year-round supply of right-sized lumpfish juveniles. This is a pilot study for a more comprehensive experiment which is recently completed and analysis of the data is pending.

A combination of short photoperiod and higher temperature could provide better management tool in controlling the gonadal maturation and synchronized spawning in lumpfish.
Ultrasound can be used to identify the gender of lumpfish from juveniles (less than 50 g) to adults which is very important tool in maintaining a proper sex ratio in breeding programs.

References

Davie, A., Mazorra de Quero, C., Bromage, N., Treasurer, J., Migaud, H., 2007. Inhibition of sexual maturation in tank reared haddock (*Melanogrammus aeglefinus*) through the use of constant light photoperiods. Aquaculture 270, 379–389.

Imsland, A.K., Hangstad, T.A., Jonassen, T.M., Stefansson, S.O., Nilsen, T.O., Hovgaard, P., Elvegård, T.A., Lindberg, K.S., Mikalsen, B., Urskog, T.C., Norberg, B., Andersson, E., Spetland, F., Reynolds, P., 2019. The use of photoperiods to provide year round spawning in lumpfish *Cyclopterus lumpus*. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 228, 62–70.

Imsland, A.K., Jonassen, T.M., Hangstad, T.A., Stefansson, S.O., Elvegård, T.A., Lemmens, S.C.A., Urskog, T.C., Nytrø, A. V., Reynholds, P., 2018. The effect of continuous light and compressed photoperiods on growth and maturation in lumpfish *Cyclopterus lumpus*. Aquaculture 485, 166–172.

Tveiten, H., Johnsen, H., 1999. Temperature experienced during vitellogenesis influences ovarian maturation and the timing of ovulation in common wolffish. J. Fish Biol. 55, 809–819.

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