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The Suitable Temperature to Stimulate the Immuno-Endocrine Interaction and Growth Improvement of *Oreochromis niloticus* Larvae

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ABSTRACT

The adrenocorticotropic homone (ACTH) and corticotropin-releasing factor (CRF) have functional roles during *Oreochromis niloticus* development. The present investigation looked at how temperature affected *O. niloticus* larval development regarding growth, survival rate, CRF and ACTH immunoreactivity, as well as mucous cell activity. Exposure to temperature enhanced larval growth, as demonstrated by the higher increases in terms of larval weight and length observed during the experiment at 30 and 35°C. Furthermore, compared to the group under control, the contact with these temperatures produced a noticeably higher larval survival rate. Temperature-exposed *O. niloticus* larvae produced more CRF in the fully formed stomach; the mucosal layer's synthetic and secretory activities increased, as evidenced by a strong immunoreaction and the existence of secretory vacuoles in CRF-immunoreactive cells. In addition, goblet cells within the growing intestine of treated larvae exhibited increased levels of both the quantity and strength of ACTH immunoreactivity. Furthermore, temperature exposure influences mucous cell activity. Mucous cell count in the alimentary canal increased after exposure to the previously mentioned environmental temperatures. In addition, qualitative alterations were demonstrated in the composition of mucus, which changed from being primarily neutral to having acid mucosubstances or a mix of acid and neutral mucosubstances. Hence, increased temperature-induced advanced digestive function improves immune-endocrine interaction, food intake during the critical period of initial feeding, primary stress response. Additionally, it enhances the protective functions of mucins, CRF and ACTH. Consequently, growth is increased and the mortality rate is lowered. These data imply that contact with the ideal temperature range of 30 to 35°C may be helpful for the spawning and growth of *O. niloticus* fingerlings.

INTRODUCTION

Growth and fish larvae survival are mostly reliant on their ability to feed, digest their food, and take in nutrients. Since it affects their later survival and growth, fish larvae first feeding time is viewed as crucial **(Ranjan *et al*., 2018; Sakyi *et al*., 2021)**. Studies comparing the early development of various teleost larvae have demonstrated that the period of yolk absorption corresponds with the differentiation and development of

some organs, particularly the digestive tract **(Fujimura & Okada, 2007; Mousa *et al*., 2024)**.

The raising of the Nile tilapia (*O. niloticus*) in the Egyptian hatcheries has given rise to sporadic and unpredictable fry production. Raising larvae has proven to be very challenging, with significant mortality rates typically occurring in the age of 7- 21 days following hatching **(Cuevas-Rodríguez *et al*., 2017; Ali *et al*., 2020)**. Given that starvation results from the physical inability to eat after endogenous reserves are exhausted, the cause of these deaths may be physiological in nature **(Ranjan *et al*., 2018; Sakyi *et al*., 2021)**. Low survival in the larviculture, however, can also be linked to additional variables including poor environmental conditions, ignorance of their ontogeny, inadequate nutrition in fish diets, and unknown digestive capacity **(Nakauth *et al*., 2016; Neumann *et al*., 2020)**. Warmer temperatures typically lead to greater food consumption, improved metabolic function, and faster fish larval growth **(Fontaine, 2008; Tine *et al*., 2022)**. The growth, survival, immune systems, activities of digestive and antioxidant enzymes, and metabolites of juvenile fish are all impacted by water temperature **(Soriano *et al*., 2018, Li *et al*., 2023)**. The main biological interface that fish have with the aquatic environment is the epidermal mucus layer. Fish mucus coats serve as the main line of defense against infection **(Pickering, 1974; Pickering & Macey, 1977; Neumann *et al*., 2020)**. Additionally, mucus is crucial to several food processing activities **(Kozarić *et al*. 2007; Diaz *et al*. 2008; Okuthe & Bhomela, 2021; Bosi *et al*., 2022)**.

The gut of *O. niloticus* has been shown to contain CRF- and ACTH-immunoreactive cells since day 0 post-hatching **(Mousa *et al*., 2024)**. According to this research, these peptides may be crucial for defense mechanisms, osmoregulation, and neuroendocrine integration during development. Additionally, the CRF system may possibly lead to teleost feeding regulation **(Qi *et al*., 2019, 2020)**. Furthermore, CRF is considered the main stimulating factor and is required for the release of ACTH during the stress response for the purpose of stimulating the adrenal gland's release of glucocorticoids **(Flik *et al*., 2006; Lai *et al*., 2021; Du *et al*., 2023)**. Moreover, cortisol, the primary interrenal steroid hormone in teleosts, has been thouroughly examined for its role in a number of physiological processes, including digestibility, osmoregulation, stress response, and carbohydrate metabolism **(Clarke & Hirano, 1995; McCormick, 1995; Mancera & McCormick, 1999; Pfalzgraff *et al*., 2021)**.

This study aimed to investigate the potential impact of an ambient temperature regarding the development of *O. niloticus* larvae, specifically in relation to its effects on mucus cell activity, ACTH and CRF immunoreactivity in addition to larval growth and survival.

MATERIALS AND METHODS

Study location

The present investigation was conducted from the first day of January 2022 to September the 30th, 2022 at El-Matareyya Aquatic Resources Research Station.

Spawning and larval production

To ensure a high-quality and sufficient number of eggs, brood stocks were stocked in two ponds prior to spawning. The two sexes were kept apart in separate ponds since January and were fed a diet containing 40% crude protein every day. We used 150–250g of a medium-sized tilapia brood stock. In hapas designated for spawning, the semi-natural spawning took place on May 1st (temperature: 23– 25°C). 30 fine-mesh 1-m2 spawning hapas were stocked with brood *O. niloticus* at a ratio of two males and four females per hapa. Every day, the breeding process was observed. Following the breeding process, the fertilized eggs were gathered, placed into plastic funnels for hatching, and given approach to a running water stream.

The design of the experiment

Three groups were created from newly hatched larvae (three aquaria for every group). Each aquarium had roughly 500 larvae, or 10 larvae per liter. The first group (control) was reared at an ambient temperature (23– 25°C) lacking any treatment. Two other groups for temperatures of either 30 or 35°C were utilized. The temperature treatment lasted for thirty five days. Every day, the aquaria were cleaned, the water was changed, and the dead larvae were taken out after being counted. Pressurized air was utilized to gently aerate the water. Furthermore, an ambient photoperiod was utilized to maintain all aquariums. Natural food for the larvae was the fresh plankton collected from a fertilized pond using a plankton net.

Processing and sampling of larvae

The standard weight (W) and length (SL) of larvae for each group were taken at hatching, 2, 4, 7, 14, 21, 28, and 35 days old. An arbitrary sample of twenty larvae was taken. The larvae were measured and weighed individually after being anesthetized and dried from water using paper towels. When the experiment was over, the total average length and weight for every group and the survived number of larvae was calculated.

The larvae were anesthetized in a clove oil solution (40mg/ l) before *in toto* fixation in Bouin's fixative for 48 hours at room temperature to facilitate histological examination. After fixation, the specimens were moved to 70% alcohol and dehydrated using various ethanol solutions with different grades. At that time, they were cleaned in xylene before embedding in paraplast (M.P. 56– 58˚C). Serial transverse and longitudinal sections, each measuring 5µm in thickness, were then cut and placed on glass slides. Alcian blue (AB)/Periodic Acid-Schiff Reagent (PAS) pH 1.0 (**Pearse, 1949; Bancroft & Cook, 1984**) was used to stain a subset of the serial sections' slides for the histochemistry of mucous cells. When mucous cell histochemistry was performed at AB/PAS pH 1.0, the positive reactions for acid mucins were blue or blue greenish, for

neutral mucins, they were red, and for a combination of neutral and acid mucins, reddish purple.

Technique for immunohistochemistry

Antibodies

The National Institutes of Health provided rabbit antiserum against human ACTH. Dr. Nigel Brooks (MRC Reproductive Biology Unit, Centre for Reproductive Biology, Edinburgh, Scotland) generously donated rabbit anti-ovine CRF.

Immunohistochemical reactions

As previously mentioned **(Mousa & Mousa, 1999)**, vectastain ABC (Avidin-biotin peroxidase complex) Kit (Vector Laboratories) was typically used for immunohistochemical staining. Sections underwent a brief process that involved deparaffinization in xylene, rehydration using graded ethanol, and two 10-minute washings in phosphate-buffered saline (PBS; pH 7.4). Every incubation was carried out at 4°C, and each step was followed by washing in PBS. The sections underwent an incubation at 4°C for an entire night, with the primary antibodies at dilutions of 1:500 for ACTH and 1:1000 for CRF. Next, the sections were incubated with Vector Laboratories' biotinylated secondary antibody for 60 minutes, and for forty-five minutes with avidin-biotin-conjugated peroxidase. After that, the sections were cleaned before staining (3- 5 minutes) using 3, 3-diaminobenzidine tetrahydrochloride (DAB) (Sigma) with 0.01 percent H2O2 in 0.05 M Tris-buffered saline (pH 7.6). The sections underwent a series of procedures following the enzyme reaction: tap water washing, alcohol dehydration, xylene clearing, and DPX mounting.

Statistical analysis

The statistical program Statistical Package (SPSS) for Social Sciences was utilized in the data processing. The "t" test for paired samples was employed to contrast the means. $P< 0.05$ was the threshold for the recognized statistical significance.

RESULTS

I. Temperature effect on larval growth and survival

i) Growth in length

According to the findings depicted in Table (1), the growth of larvae was enhanced when exposed to temperature of 30°C for 35dph. Furthermore, as Table (1) illustrates, treatment having a high temperature (35°C) brought about a noticeably higher rise in larvae length compared to both low and control temperatures.

ii) Growth in weight

As shown in Table (2), the larvae exposed to 30°C had a considerably higher average body weight compared to the group under control (ambient temperature). Additionally, an elevated temperature-exposure (35°C) resulted in significantly higher values of larval body weight than a low temperature-exposure or a control temperature (Table 2).

Table 1. Effect of exposure for temperature ($Temp_1$ 30°C or $Temp_2$ 35°C), for 35 days post hatching, on total length (mean±SD mm) of *O. niloticus* larvae

Time after hatching (day)	Treatment		
	Control	$Temp_1$	$Temp_2$
0	8.0±0.06	8.0±0.07	8.0±0.07
2	8.4±0.05	8.5±0.05	8.5±0.05
4	8.9±0.06	9.0±0.10 [a]	9.0±0.1[a]
7	9.3±0.06	9.4±0.07 [a]	9.6±0.08 [a]
14	12.5±0.67	13.0±0.23 [a]	14.0±0.63 [a]
21	16.0±0.28	17.0±0.67 [a]	20.0±0.67 [a]
28	24.0±1.29	28.0±1.75 [a]	32.0±1.79 [a]
35	31.5±7.34	36.0±1.38 [b]	41.8±0.72 [a]

a: Significant differences when compared to control ($P< 0.005$).
b: Significant differences when compared to control ($P< 0.05$).

Table 2. Effect of exposure for temperature ($Temp_1$ 30°C or $Temp_2$ 35°C), for 35 days post hatching, on total weight (mean±SD mm) of *O. niloticus* larvae

Time after hatching (day)	Treatment		
	Control	$Temp_1$	$Temp_2$
0	10.0±0.28	10.0±0.29	10.0±0.29
2	10.8±0.13	11.0±0.11 [a]	11.0±0.11[a]
4	12.0±0.40	13.0±0.36 [a]	13.0±0.36[a]
7	14.0±0.46	15.3±0.20 [a]	15.8±0.20 [a]
14	28.0±1.72	36.1±1.74 [a]	37.0±1.92 [a]
21	110±6.64	160±9.18 [a]	190±9.87 [a]
28	260±10.13	320±17.40 [a]	450±20.20 [a]
35	530±26.56	725±47.74 [a]	1000±70.45 [a]

a: Significant differences when compared to control ($P< 0.0005$).

iii) Survival rate

Table (3) presents data indicating that larval rearing was enhanced by 30°C temperature exposure, as demonstrated by low mortalities noticed throughout the 35-day treatment period. The temperature-exposed larvae showed a noticeably higher survival rate in comparison to the group under control. Furthermore, compared to low temperatures, exposure to temperature of 35°C produced a noticeably reduced survival rate. According to Table (3), the average survival rates for larvae subjected to lower, higher, and control temperatures were 80, 76.5, and 40.7%, respectively.

Table 3. Effect of exposure for temperature ($Temp_1$ 30°C or $Temp_2$ 35°C), for 35 days post hatching, on survival rate (%) of *O. niloticus* larvae

Time after hatching (day)	Treatment		
	Control	$Temp_1$	$Temp_2$
7	95±0.28	95±1.05	94±1.28
14	70.2±1.21	87.9±1.37 [a]	85.2±1.38 [a]
21	50.6±1.26	85±1.39[a]	77.5±1.25 [a]
28	45.5±1.21	80±1.53 [a]	76.6±1.62 [a]
35	40.7±1.20	80±1.53 [a]	76.5±1.40 [a]

a: Significant differences when compared to control ($P< 0.005$).

II. Temperature effect on CRF and ACTH immunoreactivity

Using immunohistochemistry, the distribution of CRF and ACTH immunoreactivity was examined in developing the larvae of *O. niloticus* from all experimental groups between 0 and 28dph. The immunohistochemical analysis indicated that the developing stomach was the primary site of CRF immunoreactivity, while the intestine was the only site of ACTH immunoreactivity (Figs. 1- 4). CRF immunoreactivity in the stomach was limited to the mucosal epithelium (Figs. 1- 4). However, developing intestinal goblet cells showed ACTH immunoreactivity. (Fig. 2c, f).

Temperature exposure for *O. niloticus* larvae increased the CRF secretion, as evidenced by the degranulated appearance of CRF-ir cells (Fig. 1e, f). The larvae exposed to temperature had a more developed stomach than the control group at the same stage of growth (Fig. 1). In the well-developed stomach, a strong immunoreaction with CRF was seen after four days of being exposed to low temperatures in contrast to the group under control (Fig. 2a, b, d, e). Furthermore, in temperature-exposed larvae, ACTH-ir cells demonstrated a rise in both quantity and immunoreactivity (Fig. 2c, f). The well-developed stomach of larvae exposed to temperature (30 °C) for 15 days showed an increased secretory activity of the CRF-ir cells, as demonstrated by the vacuolated appearance of immunoreactive cells in multiple folds of the mucosal layer (Fig. 3a- f). At 28dph, a further rise in the CRF production was noted in the larvae exposed to temperature (Fig. 4a- f). Strong immunoreaction and the presence of secretory vacuoles in CRF-ir cells indicate that the synthetic and secretory activities were enhanced in the mucosal layer of the multiple folds in the stomach of temperature-exposed larvae (Fig. 4d, f).

III. The impact of ambient temperature on the activity of mucous cells

Several mucous cells are distributed in the esophageal region of *O. niloticus* during its larval development (Fig. 5a, b). The majority of these cells exhibit positive staining for both periodic acid Schiff (PAS) and alcian blue (AB). Temperature exposure

has an impact on mucous cell activity in larvae (Fig. 5d, e). At 35dph, size and quantity of mucous cells in the esophageal region of temperature-exposed larvae increase. While few of these cells are weakly stained with PAS, most of them are strongly positive for AB (Fig. 5e). Additionally, the quantity and size of intestinal mucosa mucous cells of larvae were increased after 35 days of exposure to temperature. In comparison to control larvae, these cells exhibit a stronger PAS stain (Fig. 5c, f).

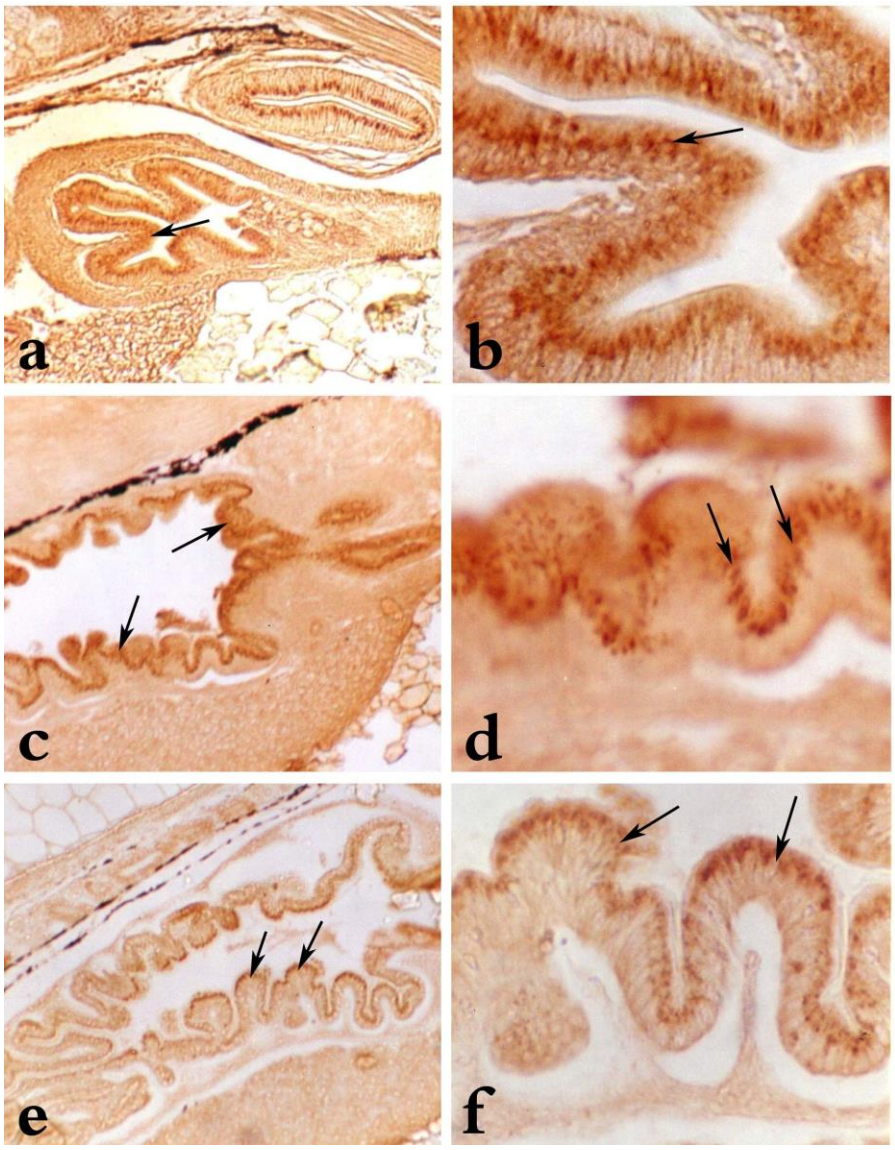

Fig. 1. The sagittal *O. niloticus* sections of larvae were immunostained using a rabbit polyclonal antibody against ovine CRF. (a, c and e) X100, (b, d and f) X400. Only the mucosal epithelium (arrows) of the stomach (S) exhibited CRF immunoreactivity. **(a)** and **(b)** Larvae from the control group 36 hours after hatching. **(c)** and **(d)** Larva from 30°C exposed group 36 hours after hatching. Remarkably, the group exposed to 30°C showed increased production of CRF. **(e)** and **(f)** Larva from the group exposed to 35°C 36 hours after hatching. The group exposed to 35°C showed a rise in CRF secretory activity

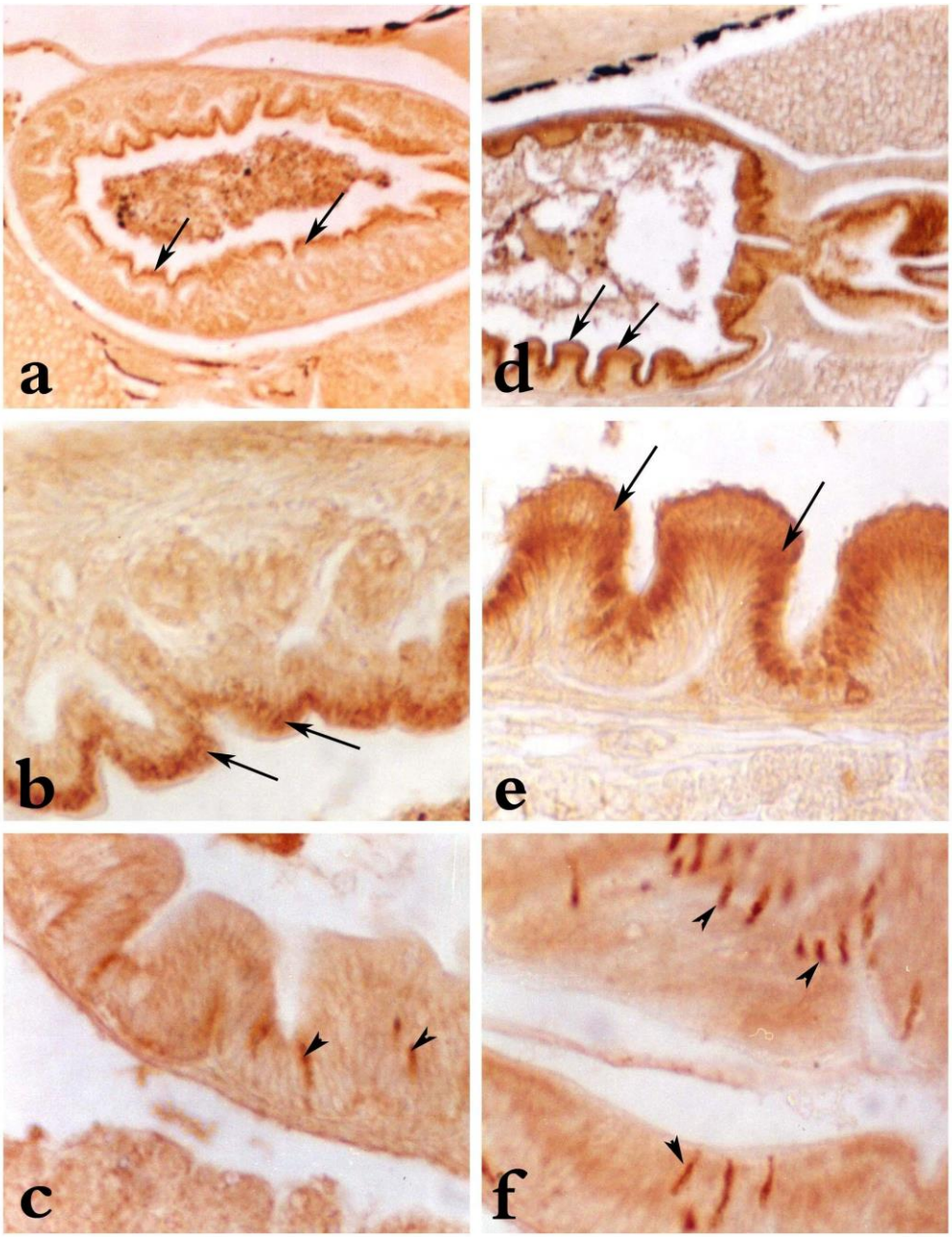

Fig. 2. *O. niloticus* larvae's sagittal sections were immunostained with rabbit polyclonal antibodies against ovine CRF **(a**, **b**, **d**, and **e)** or rabbit antibodies against human ACTH **(c** and **f)**. **(a** and **d)** X100, **(b**, **c**, **e** and **f)** X400. The mucosal epithelium (arrows) of stomach (s) exhibited CRF immunoreactivity, whereas the developing intestine's goblet cells (arrowheads) displayed ACTH immunoreactivity. **(a), (b)** and **(c)** Larva from control group; that are four days old. **(d)**, **(e)** and **(f)** 4 days-old larva exposed to 30°C. The group that was exposed to 30°C indicated an increase in both CRF and ACTH production

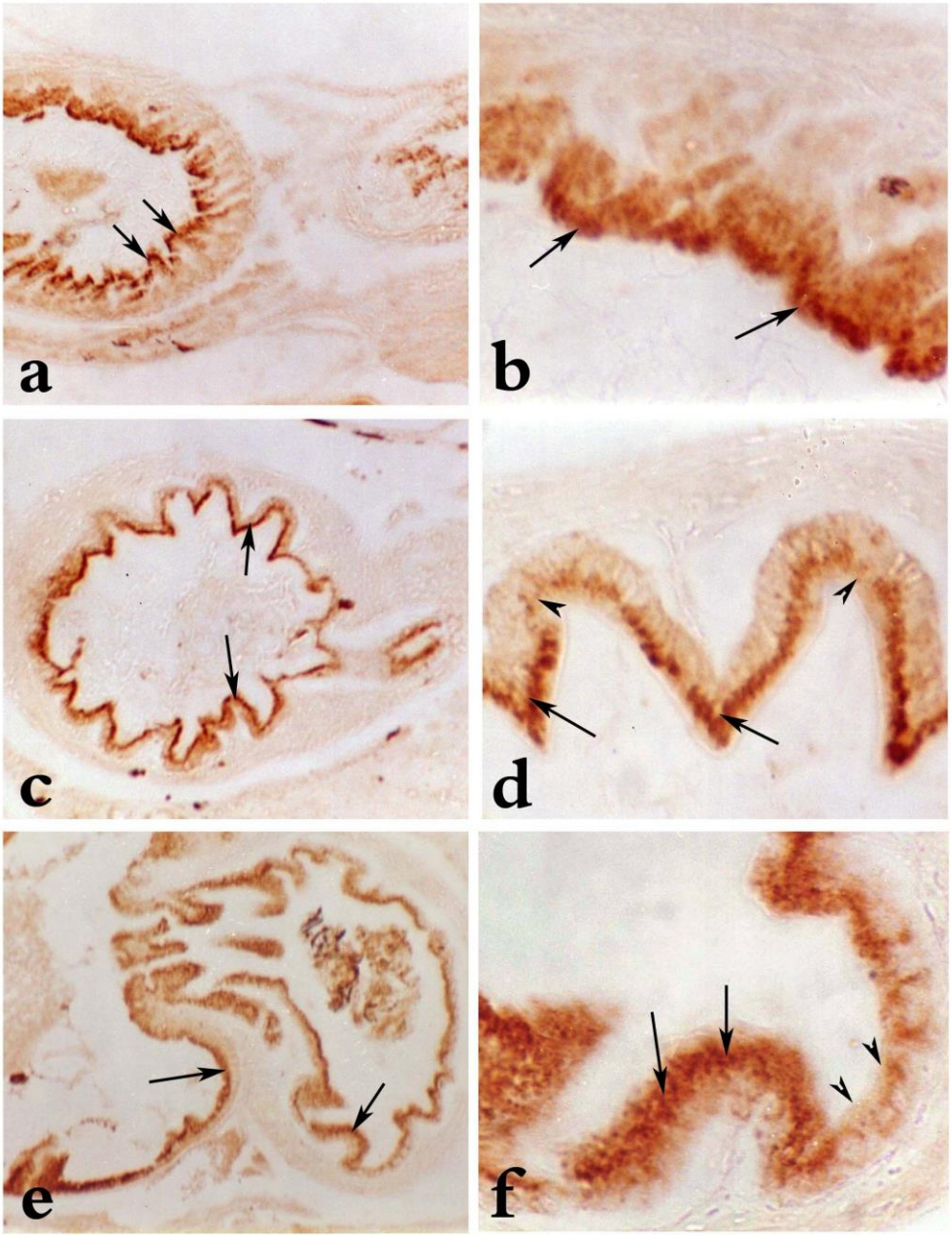

Fig. 3. 15-day-old *O. niloticus* larvae's sagittal sections were immunostained with a rabbit polyclonal antibody against ovine CRF. **(a**, **c** and **e)** X100, **(b**, **d** and **f)** X400. Only the mucosal epithelium (arrows) of the stomach (S) exhibited CRF immunoreactivity. **(a)** and **(b)** Larva from control group. **(c)** and **(d)** Larvae from the group exposed to 30°C. **(e)** and **(f)** Larva from 35°C-exposed group. The well-developed stomach of temperature-exposed larvae exhibited enhanced secretory activity of CRF-ir cells, as indicated by the vacuolated appearance of immunoreactive cells (arrowheads) in the multiple folds of the mucosal layer **(c-f)**.

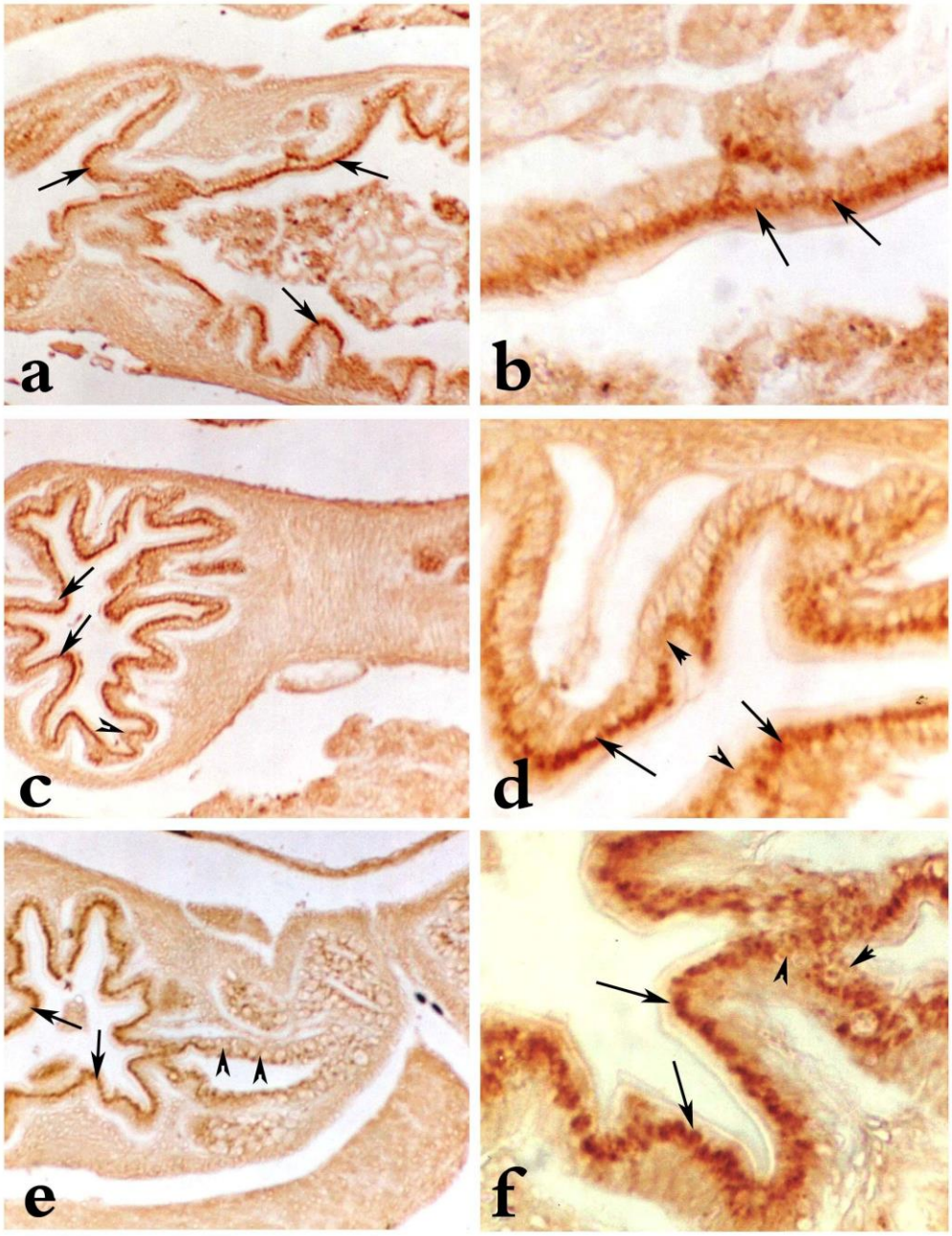

Fig. 4. Sections of the sagittal plane of 28-day-old *O. niloticus* larvae immunostained against ovine CRF using a rabbit polyclonal antibody. **(a**, **c** and **e)** X100, **(b**, **d** and **f)** X400. CRF immunoreaction was obtained in the mucosal epithelium (arrows) of the stomach (S). **(a)** and **(b)** Larva from control group. **(c)** and **(d)** 30°C-exposed group larva. **(e)** and **(f)** Larva from 35°C-exposed group. Strong immunoreaction (arrows) and the existence of secretory vacuoles (arrowheads) in CRF-ir cells (c-f) indicate that both the synthetic and secretory activities were increased in the mucosal layer of the numerous folds in the stomach of treated larvae

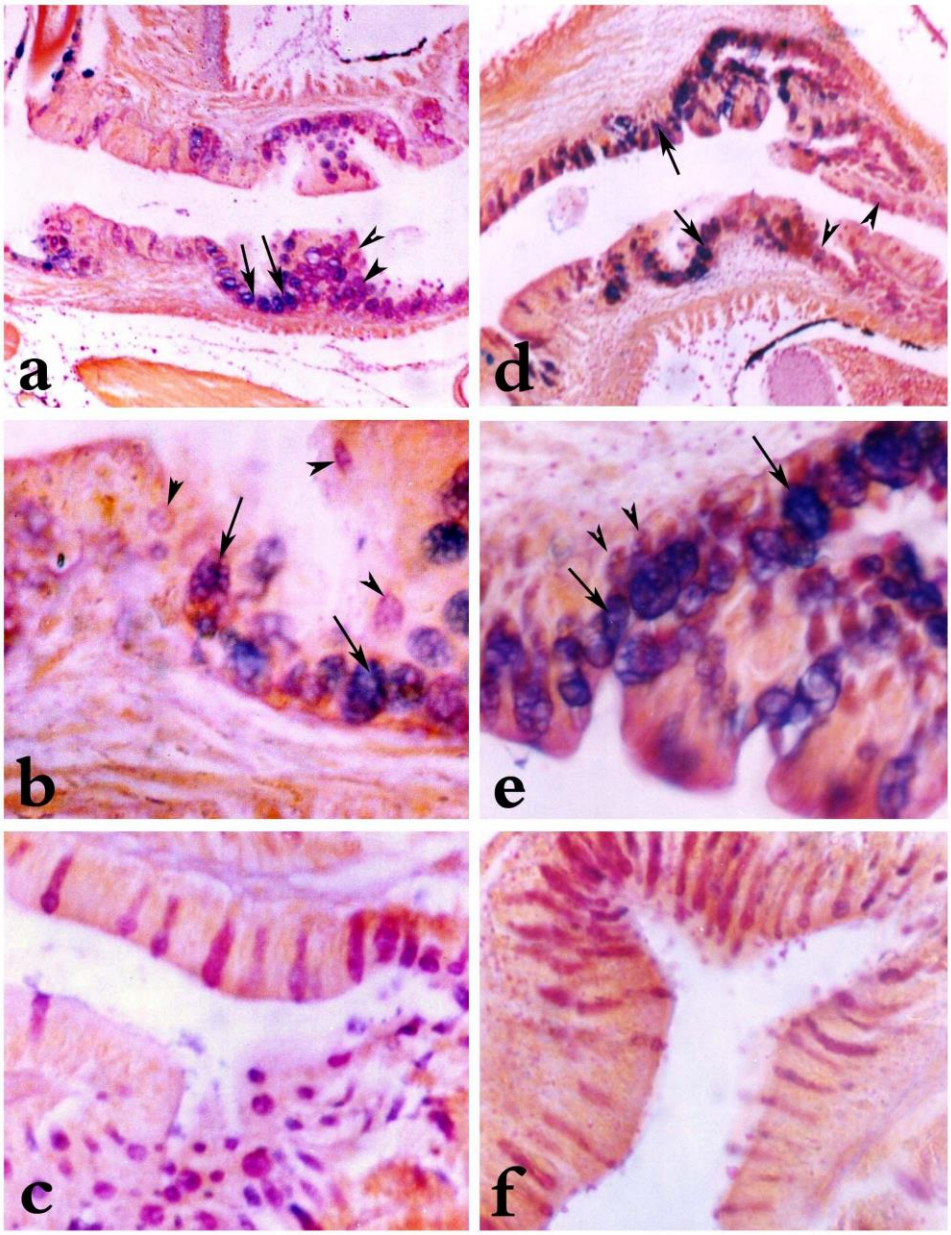

Fig. 5. The distribution of digestive tract mucous cells displayed in sagittal sections of *O. niloticus* larvae, at 35dph, stained with AB-PAS-OG. **(a** and **d)** X100, **(b**, **c**, **e** and **f)** X400. **(a)** and **(b)** Esophageal region of control group larvae. The mucous cells exhibit weak staining with both PAS (arrowheads) and AB (arrows). **c)** Intestine of control group larvae. Numerous mucous cells are present, have a goblet shape, and possess a positive PAS stain. **(d)** and **(e)** Esophageal region of 30°C-exposed group larvae. The size and number of mucous cells in the temperature-exposed larvae increase; most of these cells exhibit strong AB (arrow) staining, while a small percentage also exhibit PAS (arrowhead) staining. **f)** Intestine of 30°C-exposed group larvae. Observe that the temperature-exposed group's mucous cells are abundant and highly PAS-stained

DISCUSSION

In an earlier paper **(Mousa *et al*., 2024)**, CRF- and ACTH-immunoreactive cells were observed in the *O. niloticus* gut from day 0 post-hatching (dph). Considering this study, these peptides may be crucial for defense mechanisms, osmoregulation, and neuroendocrine integration during development. In the current investigation, we looked at how temperature affects the behaviors of CRF, ACTH, and mucus-secreting cells during *O. niloticus* larval development, and how that affects the growth and survival of larvae.

Temperature-exposed *O. niloticus* larvae exhibited an increase in the number of CRF cells produced in the developed stomach. Strong immunoreaction and the presence of secretory vacuoles in CRF-immunoreactive cells indicate that the synthetic and secretory activities were increased in the mucosal layer of numerous folds in the stomach of the treated larvae. CRF possibly involved in controlling teleost feeding **(Qi *et al*., 2019, 2020)**. Furthermore, CRF is the principal neurohormone regulating ACTH secretion in the stress reaction, which causes glucocorticoids to be secreted from the adrenal glands **(Lai *et al*., 2021; Du *et al*., 2023)**. The current data provide a strong support for this, as ACTH immunoreactivity in the goblet cells of the developing intestines demonstrated a rise in the quantity and strength of immunoreaction in temperature-exposed larvae. Cortisol, the principal interrenal steroid hormone in teleosts, has been thoroughly researched for its function in a number of physiological functions, such as carbohydrate metabolism, stress response, digestibility, and osmoregulation **(McCormick, 1995; Mancera & McCormick, 1999; Pfalzgraff *et al*., 2021)**.

It is worthy to mention that CRF and ACTH are among the primary players in the stress response **(Mola *et al*., 2004)**. The identification of CRF- and ACTH-like materials by immunohistochemistry in the same digestive tract regions of *O. niloticus* where the gut-associated lymphoid tissue (GALT) will differentiate, raising the possibility that CRF and ACTH are involved in early defense mechanisms in *O. niloticus*, prior to the emergence of immune responses mediated by cells in GALT. A greater primitive function in vertebrates for this peptide, according to similar findings, may be as a dual hypophysiotropic agent acting on both the interrenal (adrenal) and thyroid axes **(Boorse & Denver, 2004)**. Thyroid hormones are known to raise the survival and hasten the immune system's development in the teleost larvae **(Gavlik *et al*., 2002; Mousa *et al*., 2002; Lam *et al*., 2005)**. This corresponds to the existing findings because the increased production of the CRF following temperature exposure (30°C) may enhance the thyroid hormone secretion and strengthen the survival and growth rates of larvae exposed to temperature.

The provided data here point to the importance of CRF and ACTH in immune response, food consumption, and growth during *O. niloticus* development. Accordingly, larval growth and survival are increased by temperature exposure. Increased food intake, better metabolic performance, and quicker growth of fish larvae are typically connected to warmer temperatures **(Fontaine, 2008; Tine *et al*., 2022)**. Water temperature affects

the juvenile fish growth, survival, immune systems, digestive, and action of metabolites and antioxidant enzymes **(Soriano *et al*., 2018, Li *et al*., 2023)**.

The ability and desire of fish to eat, additionally how they digest food, absorb nutrients within the digestive system, and store excess energy, are all influenced by temperature **(Volkoff & Rønnestad, 2020)**. Compared to the control treatment, the prolonged temperature elevation improved lipid digestion. However, growth performance was compromised though this did not seem to be related with the increased plasma cortisol levels **(Pfalzgraff *et al*., 2021)**. Chronically high plasma cortisol levels have an atrophic effect on digestive tissues, which impairs nutrient absorption, reduces metabolizable energy available, and increases routine energy expenditure, all of which raise the cost of growth **(Pfalzgraff *et al*., 2021)**.

Mucous cell activity is also impacted by the temperature to which larvae of *O. niloticus* are exposed. During the temperature exposure, mucous cells multiplied in the alimentary canal, and the combination of the mucus changed qualitatively. Similar results were recorded in larvae of *Cyprinus carpio* **(Mousa *et al*., 2002)**. The epidermal mucous layer serves as fish's primary biological interface with the water. Fish mucus coats are the primary defense mechanism against pathogens **(Pickering & Macey, 1977; Neumann *et al*., 2020)**. Additionally, mucus is essential for several food processing activities **(Diaz *et al*. 2008; Okuthe & Bhomela, 2021; Bosi *et al*., 2022)**.

Finally, the effects of environmental temperature on CRF and ACTH immunoreactivity have been noted during the development of *O. niloticus* gut. It was deduced that these peptides may have significant roles during development, including neuroendocrine integration, food intake, early defense mechanisms, and growth. Additionally, the significance of temperature during the *O. niloticus* larval rearing has emerged.

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Conflict of Interest

The authors state that there aren't any conflicts of interest.

REFERENCES

Ali, A.; Moustafa, Y. T. and El-Said, S. (2020). Evaluating the influence of different water sources on water quality, survival and growth rates of Nile tilapia (*Oreochomis niloticus*) larvae in tilapia hatcheries. Egyptian Journal for Aquaculture, 10 (1): 45-64.

Bancroft, J.D. and Cook, H.C. *Manual of histological techniques*. New York: Churchill Livingstone; 1984.

Boorse, G.C. and Denver, R.J. (2004). Expression and hypophysiotropic actions of corticotropin-releasing factor in *Xenopus laevis*. Gen. Comp. Endocrinol., 137: 272-282.

Bosi, G.; Merella, P.; Maynard, B. J. and Sayyaf Dezfuli, B. (2022). Microscopic Characterization of the Mucous Cells and Their Mucin Secretions in the Alimentary Canal of the Blackmouth Catshark *Galeus melastomus* (Chondrichthyes: *Elasmobranchii*). Fishes, 7 (1), 8.

Clarke, W.C. and Hirano, T. (1995). Osmoregulation. In *"Physiological Ecology of Pacific salmon"* (Groot, C.; Margolis, L. and Clarke, W. C., Eds.), *pp*: 317-377. UBC press, Vancouver.

Cuevas-Rodríguez, B. L.; García-Ulloa, M.; Hernández-Llamas, A.; Racotta, I.; Valdez-González, F. J.; Polanco-Torres, A. and Rodríguez-González, H. (2017). Evaluating quality of Nile tilapia (*Oreochromis niloticus*) eggs and juveniles from different commercial hatcheries. Latin american journal of aquatic research, 45 (1): 213-217.

Díaz, A.O.; García, A.M. and Goldemberg, A.L. (2008). Glycoconjugates in the mucosa of the digestive tract of *Cynoscion guatucupa*: a histochemical study. Acta Histochemica 110 (1): 76–85. https://doi.org/10.1016/j.acthis.2007.08.002

Du, R.; Shi, X.; Chen, F.; Wang, L.; Liang, H.; Hu, G. (2023). Corticotropin-Releasing Hormone: A Novel Stimulator of Somatolactin in Teleost Pituitary Cells. Cells 2023, 12, 2770. https://doi.org/10.3390/cells12242770

Flik, G.; Klaren, P. H.; Van den Burg, E. H.; Metz, J. R. and Huising, M. O. (2006). CRF and stress in fish. General and comparative Endocrinology, 146 (1): 36-44.

Fontaine, L.P. (2008). Interaction of Temperature, Dissolved Oxygen and Feed Energy on Ecophysiological Performance of Juvenile Red Drum. PhD Thesis, Office of Graduate Studies of Texas A & M University, 161.

Fujimura, K. and Okada, N. (2007). Development of the embryo, larva and early juvenile of Nile tilapia *Oreochromis niloticus* (Pisces: Cichlidae). Developmental staging system. Development, growth & differentiation, 49 (4): 301-324.

Gavlik, S.; Albino, M. and Specker, J.L. (2002). Metamorphosis in summer flounder: manipulation of thyroid status to synchronize settling behavior, growth, and development. Aquacult. 203: 359-373.

Kozarić. Z.; Kužir, S.; Petrinec, Z.; Gjurćević, E. and Baturina, N. (2007). Histochemistry of complex glycoproteins in the digestive tract mucosa of Atlantic Bluefin tuna (*Thynnus* L.) Veterina- riski Arhiv 77 (5): 441–452.

Lai, F.; Royan, M. R.; Gomes, A. S.; Espe, M.; Aksnes, A.; Norberg, B.; Gelebart, v. and Rønnestad, I. (2021). The stress response in Atlantic salmon (*Salmo salar* L.): identification and functional characterization of the corticotropin-releasing factor (crf) paralogs. General and comparative endocrinology, 313, 113894.

Lam, S.H.; Sin, Y.M.; Gong, Z. and Lam, T.J. (2005). Effects of thyroid hormone on the development of immune system in zebrafish. Gen. Comp. Endocrinol., 142: 325-335.

Li, R. X.; Amenyogbe, E.; Lu, Y.; Jin, J. H.; Xie, R. T. and Huang, J. S. (2023). Effects of low-temperature stress on intestinal structure, enzyme activities and metabolomic analysis of juvenile golden pompano (*Trachinotus ovatus*). Frontiers in Marine Science, 10, 1114120.

Mancera, J.M. and McCormick, S.D. (1999). Influence of cortisol, growth hormone, insulin-like growth factor I and 3,5,3′-triiodo-L-thyronine on hypoosmoregulatory ability in the euryhaline teleost *Fundulus heteroclitus*. Fish Physiol. Biochem. 21: 25-33.

McCormick, S. D. (1995): Hormonal control of gill Na^+ /K^+-ATPase and chloride cell function. *In*: *Fish Physiology*. Vol. *14*: 285-315. Edited by C. M. Wood and T. J. Shuttleworth. New York, Academic Press.

Mola, L.; Bertacchi, I.; Gambarelli, A. and Pederzoli, A. (2004). Occurrence of ACTH- and enkephalin-like peptides in the developing gut of *Dicentrarchus labrax* (L.). Gen. Comp. Endocrinol., 136: 23-29.

Mousa, M.A.. and Mousa, S.A. (1999). Immunocytochemical study on the localization and distribution of the somatolactin cells in the pituitary gland and the brain of *Oreochromis niloticus* (Teleostei, Cichlidae). Gen. Comp. Endocrinol., 113: 197–211.

Mousa, M.A.; El-Gamal, A.S. and Khalil, M-B.A. (2002): Effect of exogenous hormonal treatment on growth, survival and mucus cells activity during larval development of common carp, *Cyprinus carpio*. J. Egypt. Ger. Soc. Zool., 37 (C): 193-207.

Mousa, M. A.; Kora, M. F.; El-Sisiy, D.M. and Khalil, N. A. (2024). Immunoreactivity of corticotrophin releasing factor (CRF) and adrenocorticotropic hormone (ACTH) in the developing digestive tract of Nile tilapia, *Oreochromis niloticus*. Egypt. J. Aquat. Biol. & Fish. , 28 (3): 677-692.

Nakauth, A.C.S.S.; Villacorta-Correa, M.A.; Figueiredo, M.R.; Bernardino, G. and França, J.M. (2016). Embryonic and larval development of *Brycon amazonicus* (Spix & Agassiz, 1829). Brazil J Biol., 76: 109–116.

Neumann, E.; Paes, M. D. C. F.; Mendes, J. M. R.; de Souza Braga, F. M. and Nakaghi, L. S. O. (2020). Ontogeny of the digestive tract of *Brycon amazonicus* (Teleostei, Bryconidae) under culture conditions: from hatching to juvenile stage. Zygote, 28 (3): 208-216.

Okuthe, G. E. and Bhomela, B. (2021). Morphology, histology and histochemistry of the digestive tract of the Banded tilapia, *Tilapia sparrmanii* (Perciformes: Cichlidae). Zoologia (Curitiba), 37, e51043.

Pearse, A.G.E. (1949). The cytochemical demonstration of gonadotropic hormone in the human anterior hypophysis. J. Pathol. Bacteriol., *61*: 195.

Pfalzgraff, T.; Lund, I. and Skov, P. V. (2021). Cortisol affects feed utilization, digestion and performance in juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 536, Article 736472. https://doi.org/10.1016/j.aquaculture.2021.736472

Pickering, A.D. (1974). The distribution of mucous cells in the epidermis of the brown trout *Salmo trutta* (L.) and the char *Salvelinus alpinus* (L.). J. Fish Biol. 6: 111-118.

Pickering, A.D. and Macey, D.J. (1977). Structure, histochemistry and the effect of handling on the mucous cells in the epidermis of the char *Salvelinus alpinus* (L.). J. Fish Biol. 10: 505-512.

Qi, J.; Tang, N.; Wu, Y.; Chen, H.; Wang, S.; Wang, B.; Xu, S.; Wang, M.; Zhang, X.; Chen, D. and Zhou, B. (2019). The transcripts of CRF and CRF receptors under fasting stress in Dabry's sturgeon (*Acipenser dabryanus Dumeril*). Gen. Comp. Endocrinol., 280: 200-208.

Qi, J.; Xu, S.; Wang, M.; Chen, H.; Tang, N.; Wang, B.; Li, Y.; Zhang, X.; Chen, D.; Zhou. B.; Zhao, L.; Wang, Y. and Li, Z. (2020). Changes in corticotropin releasing factor system transcript levels in relation to feeding condition in *Acipenser dabryanus*. Peptides, 128, 170309.

Ranjan, R.; Megarajan, S.; Xavier, B.; Ghosh, S.; Santhosh, B. and Gopalakrishnan, A. (2018). Broodstock development, induced breeding and larval rearing of Indian pompano, *Trachinotus mookalee* (Cuvier, 1832) A new candidate species for aquaculture. Aquaculture, 495: 550-557

Sakyi, M. E.; Cai, J.; Ampofo-Yeboah, A.; Anokyewaa, M. A.; Wang, Z. and Jian, J. (2021). Starvation and re-feeding influence the growth, immune response, and intestinal microbiota of Nile tilapia (*Oreochromis niloticus*; Linnaeus 1758). Aquaculture, 543, 736959.

Soriano, E. L.; Ramí rez, D. T.; Araujo, D. R.; Gómez-Gil, B.; Castro, L. I. and Sánchez, C. G. (2018). Effect of temperature and dietary lipid proportion on gut microbiota in yellowtail kingfish *Seriola lalandi* juveniles. Aquaculture. 1 (497), 269–277. doi: 10.1016/j.aquaculture.2018.07.065

Tine, M.; Thiombane, A.B.; Sonko, F.; Ndiaye, N.D. and Diadhiou, H.D. (2022). Suitable Temperature, Stocking Density and Feeding Rate for Optimal Growth of Sex Reversed Fry of Nile Tilapia *Oreochromis niloticus* (Senegal River Strain). Agricultural Sciences, 13: 897-915.

Volkoff, H. and Rønnestad, I. (2020). Effects of temperature on feeding and digestive processes in fish, Temperature, 7:4, 307-320, DOI:10.1080/23328940.2020.1765950