

## STUDY THE PERFORMANCE OF NATIVE DUCK AND FRESHWATER MUSSELS REARED IN CONCRETE POND SYSTEM

PEMA THINLEY, RENCHEN LHAMO, CHETEN DORJI AND DRUKPOLA

National Research & Development Centre for Aquaculture

\*Author for correspondence: pemathinley2@moal.gov.bt

Copyright© 2023 Pema Thinley. The original work must be properly cited to permit unrestricted use, distribution, and reproduction of this article in any medium.

**ABSTRACT:** The present study investigated the performance of Duck mussels and freshwater mussels reared in a concrete pond system. The study also identified appropriate host fish species for completing a unique reproductive cycle of the native mussels to carry glochidia during the larval stage. A total of 1200 numbers of healthy mussels – 600 each Duck mussels and freshwater mussels were stocked in the captive condition with suitable physicochemical parameters like water movement, water exchange, water temperature, dissolved oxygen, and pH. Both duck mussels and freshwater mussels were fed on natural productivity integrated with native fish species as the treatment. Mussels were provided plant-based substrate to derive natural food through a natural weathering process limiting an external feed supplementation on a trial and error basis. Variables such as total body weight, shell length, shell height, shell depth, survival rate, and host fish species were assessed. Growth indices data were analyzed using SPSS version 25.0 through a t-test to compare the various performance. The present study revealed that freshwater mussels had better growth (ADG = 0.23) and survival rate than Duck mussels (ADG = 0.07). The latter species also exhibited satisfactory growth performance under concrete pond settings. Duck mussels and freshwater mussels attained  $32.69 \pm 12.07\text{g}$  and  $112.77 \pm 33.67\text{g}$ , respectively after six months of the culture period. Both mussels had host fish species compatibility with native fishes such as Barna baril (*Barilius barna*), zebrafish or zebra danio (*Danio rerio*) and Ticto barb (*pethia ticto*) found in the Southern River system. It is evident from 90% survival rates that natural productivity adequately enabled growth performance and natural propagation. Considering the growth performance, natural propagation efficiency, and promising survival rate of the current findings, both freshwater and duck mussels qualify for food production.

**Keywords:** Duck mussel; freshwater mussel; growth performance; survival rate

### 1. INTRODUCTION

Bhutan formally started pisciculture in the early 1980s with a culture of carp along the southern belt of the country. Gradually, plan programs of the country incorporated fish as the one of food commodities with some dedicated amount of budget for enhancement of establishment across potential areas. Swiftly, the blue sector enjoyed gradual growth in the country; however, pisciculture is not that diverse in terms of farm species.

Until today, there are only six species of carp and one cold-water fish species i.e., rainbow trout (*Oncorhynchus mykiss*) for aquaculture. Aquatic species diversity is a critical and essential component. Diverse aquaculture ecosystems tend to be more productive, sustainable and possess a greater ability to withstand environmental stress like drought or invasive infestation. However, aquatic animals' diversity relative to sustainability in the country at the moment is highly questionable. To derive optimal synergistic

benefits of species biodiversity within pond ecosystems, fish farming along with other aquatic animals such as mussels, or shellfish is the choice. Such integration will enable the sustainable use of native aquatic animals' resources in the country fulfilling the conservation vision of the National Biodiversity Centre (2014). Mussel integrated with fish culture has a mutual effect in enhancing production as the mussel plays a critical role in the aquatic environment by modifying the aquatic habitat. Thus, Mussel is known as an ecosystem engineer (Borthagaray & Carranza 2007).

Duck Mussels (DM), *Anodonta anatina*, and Freshwater mussels (FWM) are large bivalves, aquatic mollusk that has compressed body enclosed within hinged shells, such as oysters, clams, mussels, and scallops. It lives in slow-flowing streams, rivers, and natural lakes. Ecologist refers to bivalve mussels as ecosystem engineers or native natural filter because they play a big role in shaping their environment. Mussels breathe and filter-feeding the water, removing dirt and pollution and allowing sunlight to a greater depth which helps oxygen-producing plants and aquatic life to depend on them. But many native freshwater mussels are in trouble and are the most imperiled group of organisms among life below water (Nobles and Zhang 2015). Their habit has been dramatically changed by the dam which alters the water temperature and how a faster deeper river flow.

Scientists are trying to help by raising the young mussels in a tank and releasing them back into the wild. Freshwater mussels have evolved a fascinating way to give their young a head start. Mama mussels have tricks to attract fish that will temporarily host glochidia in their fins or gills. Some mussels make fleshy lures that resemble crayfish or minerals. The mother releases her glochidia in a cloud, the young mussels harmoniously grow in a fish for several months till they are dropped out or burrowed into a new habitat

or in a new location as a juvenile. Other mussels like washer shells and kidney shells, package their young ones in sag that resembles a small fish to prompt real fish to snatch it up. Being a mother mussel requires passion; wait for a particular fish to harbor her offspring at the right time. Scientists are still learning about which host fish species each mussel prefers and tell us how much mussels can help restore waterways that are degraded by dams or pollution. In the future, mussels might be able to help save the rivers and streams they depend on while contributing to aquatic food production.

Currently, the national domestic production of fresh fish in 2021 was 192.97 MT (National Statistics Bureau [NSB] 2021) in contrast to 2000 MT of fish imported (Bhutan Trade Statistics 2021). Obviously, in the latest millennium, the average annual growth rate remains below 1% which is a clear indication of a poor growth rate for the aquaculture sector perhaps associated with species diversity as one of the critical components. The annual per capita consumption of fish in the country stands at 3.16 kg/person in contrast to the global per capita consumption risen over 20 kg in 2016 (FAO 2016). Thus, the enhancement of domestic fish production requires due attention to boost production to enable fish sufficiency internally curbing the huge import figures at the earliest possible. To optimize the production, aquatic animals' diversity is the vital modus operandi to incorporate into aquaculture. In this regard, the National Research & Development Centre for Aquaculture (NR&DCA), Gelephu had explored duck mussels, *A. anatina* (Linnaeus 1758), and freshwater mussels' culture to understand their growth performance and morphometric characteristics like size, shape, color including other parameters like mortality and onset of the breeding season.

In Bhutan, pisciculture has been practiced since the early 1980s but the culture of other viable aquatic animals has not appeared

under the wings of the Bhutanese aquaculture sector. Aquaculture is the farming of aquatic organisms involving technologies in the rearing process to enhance production. The transition of pisciculture to aquaculture in Bhutan is timely with a proper exploration of aquatic organisms to fill the aquatic food basket. Owing to manpower shortage, resources, technical know-how, and lack of aspiring entrepreneurs, not much attention has been given to the culture of aquatic animals. Nevertheless, the culture of aquatic animals has great potential and scope to improve the water quality for aquaculture and bring in synergy between mussels and fish to enhance production and higher economic returns to farmers.

Integrated culture of fish with mussels has a significant and mutual benefit for both fish and mussels as the pond environment is filtered from microalgae and harmful debris by the mussels during feeding. Similarly, mussels' extraordinary reproductive cycle requires specific fish host species to carry glochidia in their gills and fins to complete their larval to the juvenile stage. Thus, there is a positive ecological relationship between mussel and fish in general which ultimately enable enhanced production for life below water. However, scientific information on the morphometric performance of DM and FWM in earthen ponds although crucial is not available. Thus, this study was designed to assess the growth performance of morphometric traits such as shell size measurement (length, height, and width), wet body weight, and shape of DM and FWM for benchmark settings. Besides, the study also explored specific host fish species for the unusual reproductive life cycle, and water quality parameters required to guide mussel farming, mortality, and the onset of the breeding season for future applications.

## 2. MATERIALS AND METHODS

### 2.1 Study area

The study was conducted at the National Research & Development Centre for Aquaculture (NR&DCA), Gelephu located at 26°51.790' N and 90°31.961' E at an elevation of 252 masl. The area falls under sub-tropical climatic conditions with warm and dry winters and wet and hot summers. Annual rainfall ranges from 1500-3500 mm and temperature from 16-30°C.

### 2.2 Sampling

The centre had cohabitant two types of native mussels i.e., DM and FWM species. Small-size (usually  $\leq 3$  inches) mussels, *A. anatina* were collected from Zarkarpla River in the Zhemgang district with approval from the Department of Forests and Park Services (DoFPS). Whereas, the medium and large-size freshwater mussels were collected from the Centre's earthen ponds, inlet, and outlet canals. From the population size of 600 numbers, 240 mussels were randomly selected as a sample size for an in-depth study to record their growth indices every month (Yamane 1967). Shell length (SL) is the maximum distance between the anterior and posterior margins of the shell. Shell height (SH) is the maximum distance between the umbo or posterior wing and the ventral. Shell width (SW) is the distance measured at the thickest part of the two shell valves using Vernier calipers. The wet body weight was weighed and recorded using an electronic digital weighing balance with a precision of 0.001 g.

### 2.3 Data collection and analysis

The data on SL (cm), SH (cm), SW (cm), TBW (g), shell color, water quality parameters, host fish species, mortality, and the onset of the breeding season were collected from December 2021 till June 2022. The data collected were cleaned and processed in Microsoft Excel and then transferred to the Statistical Package for Social Sciences (SPSS) version 25.0 software for further analysis. Both descriptive and inferential statistical tests

were run to analyze the parametric data. The independent t-test was conducted to compare the mean growth indices among two species of mussels. The quantitative data on body weight, body length, and water quality parameters were analyzed using the same test.

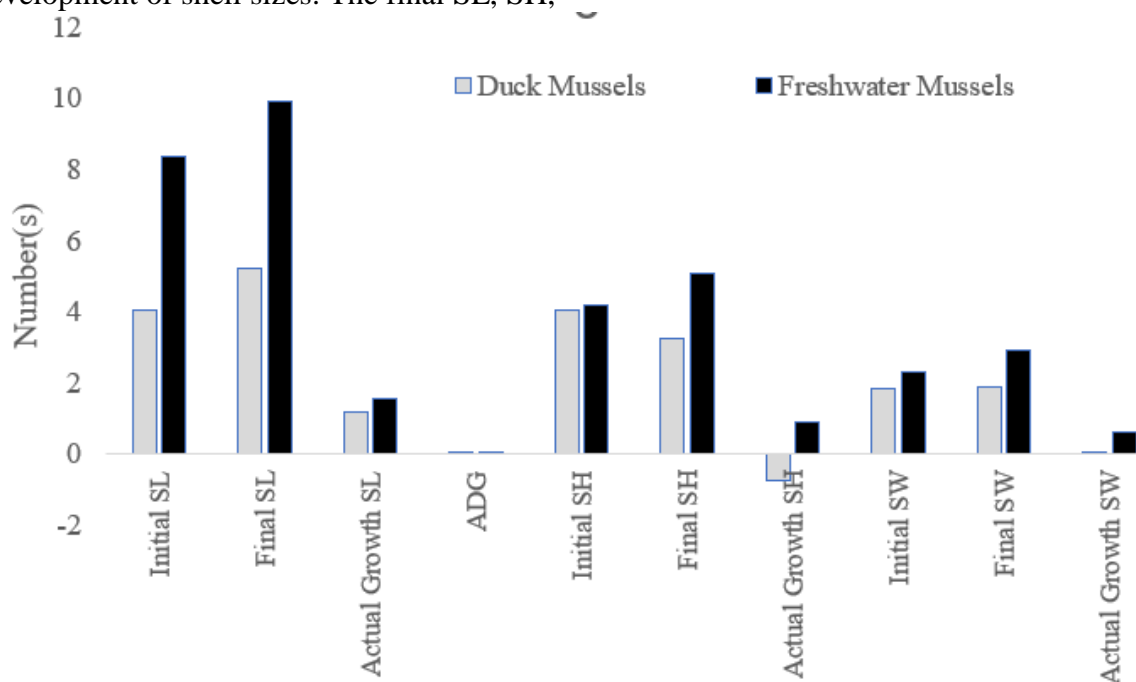
### 3. RESULTS AND DISCUSSIONS

#### 3.1 Phenotypic characteristics of mussels

##### 3.1.1 Shell sizes

Shell size in terms of SL, SH, and SW was measured and recorded during initial stocking and also during the harvest period to determine the range and strength of growth indices. For mussels, the growth is usually measured in terms of an increase in some dimensions of shell valves (Gosling 2004). There was a significant difference ( $p < 0.05$ ) in mean SL and SH except for SW ( $p > 0.05$ ) during the initial stocking between DM and FWM. The mean SL, SH, and SW measured for DM and FWM during initial stocking were  $4.03 \pm 3.44$  cm &  $8.38 \pm 1.41$  cm,  $4.02 \pm 1.25$  cm &  $4.20 \pm 0.82$  cm, and  $1.85 \pm 1.67$  cm &  $2.30 \pm 0.55$  cm respectively. After six months of the study period, the mussels were harvested and the measurements were taken to understand the development of shell sizes. The final SL, SH,

and SW recorded for DM were  $5.21 \pm 0.66$  cm,  $3.26 \pm 0.54$  cm, and  $1.88 \pm 0.33$  cm whereas FWM exhibited  $9.91 \pm 0.85$  cm,  $5.09 \pm 0.50$  cm,  $2.94 \pm 0.33$  cm growth in terms of SL, SH and SW (Figure 1). Kovitvadhni et al (2008) reported the mean shell length (mm) of  $1.83 \pm 0.74$ ,  $27.88 \pm 16.08$ , and  $49.63 \pm 6.16$  during 0-120 days, 120-270 days, and 270-360 days culture period with a shell length range of 0.34 - 78.60 mm. During the same experiment, shell height (mm) recorded were  $1.16 \pm 0.37$ ,  $12.52 \pm 6.95$ , and  $22.28 \pm 2.76$  with a range of 0.30 mm minimum and 33.44 mm maximum. Similarly, shell width (mm) recorded during 120-270 days and 270-360 days were  $5.82 \pm 3.28$  and  $10.51 \pm 1.43$  respectively. According to the Nature Spot Recording the Wildlife of Leicestershire and Rutland [NSRWLR] (2020), one of the largest freshwater duck mussels measured 75 mm to 120 mm in length which is commonly found in England. It is evident from the above findings that FWM outpaced the growth performance of DM cultured under similar conditions, thus, it is the viable native mussel candidate to prioritize for food production.



**Figure 1:** Comparative growth performance of mussels

### 3.1.2 Total body weight (TBW)

For this experiment, out of 600 numbers known population sizes, each for DM and FWM initially weighing  $20.93 \pm 10.95$  g and  $71.58 \pm 41.76$  g respectively, 240 numbers subsets were randomly selected as a sample unit (Table 1). At the end of six months culture period, DM and FWM weighed  $32.69 \pm 12.07$  g and  $112.77 \pm 33.67$  g. The DM exhibited a growth increment of 11.49 g whereas FWM had gained 41.19 g of weight revealing 0.06 and 0.20 g of average daily gain (ADG), respectively.

Based on the current findings, FWM outpaced the growth performance of DM under the same management and culture conditions of NDCA, Gelephu. It is evident to conclude that among two native species, FWM is the best choice for culture. Kovitvadhi et al. (2008) recorded TBW of  $2.41 \pm 2.10$  g and  $8.30 \pm 3.59$  g for the freshwater pearl mussel, *Hyriopsis Limnoscapha myersiana* in the 120 -270 days and 270 - 360 days culture period, respectively. The minimum TBW recorded was 0.002 g on 120 days and the maximum was 31.97 g on 360 days which is quite a higher growth rate compared to the present study. Nobles and Zhang (2015) reported that the total final mean ( $\pm$  SE) wet mass of mussels on 72 days experiment duration was  $127.57 \pm 0.58$  g,  $131.73 \pm 5.41$  g,  $113.53 \pm 2.77$  gm, and  $123,39 \pm 1.90$  g compared to their initial growth,  $124.56 \pm 4.45$  g,  $131.67 \pm 5.53$  g,  $112.08 \pm 3.02$  g and  $122.76 \pm 2.06$  g on zero (0) days respectively. The same authors observed the percentage change of  $2.58 \pm 0.58$  g,  $0.08 \pm 0.22$ g,  $1.32 \pm 0.28$  g, and  $0.50 \pm 0.40$  g for four groups of ridge mussels during a study conducted in Travis

County of Texas, USA. Mussels can acclimate and adapt to variant environmental situations owing to their phenotypic plasticity. However, culturing in polluted habitats showed lower shell growth and body weight compared to the culture performance of mussels in less polluted sites (Bonel and Lorda 2015). The highest total weight of the Mediterranean Mussel, *Mytilus galloprovincialis* was recorded in May and June which indicates optimum growth during the summer season relative to a great abundance of natural productivity (Azizi et al. 2020).

Based on the current findings, DM mussels gained 11.76 g in six months whereas FWM grew 41.19 g which is quite pronounced. When compared in terms of ADG, FWM exhibited the highest of 0.23 g. With this, the authors conclude that FWM is the best mussel performer in terms of growth performance for cultural purposes to bolster the aquatic food volume.

### 3.1.3 Shell shape and color

This study recorded a brown-black shell color for FWM and then observed an elongated elliptical shape with a concave ventral margin. The shell shape of DM is oval and swollen, shaped like an egg that wears black olive color with lively spots of green. All bivalve mollusks share the characteristic of a house made of two calcareous shells. The interior portion of the common DM has only minimal mother-of-pearl coating. The shell has an oval, rhombic form and is quite thick all the way through, with a size of up to 100mm. The scale-like external plate has a pronounced triangular

**Table 1:** Initial and final statistics on total body weight, shell sizes of two mussels

Species	Initial Data				Harvest Data			
	BW (g)	SL (cm)	SH (cm)	SW (cm)	BW (g)	SL (cm)	SH (cm)	SW (cm)
DM	$20.93 \pm 10.95^a$	$4.03 \pm 3.44^a$	$4.02 \pm 1.25^a$	$1.85 \pm 1.67^a$	$32.69 \pm 12.07^a$	$5.21 \pm 0.66^a$	$3.26 \pm 0.54^a$	$1.88 \pm 0.33^a$
FW	$71.58 \pm 41.76^b$	$8.38 \pm 1.41^b$	$4.20 \pm 0.82^a$	$2.30 \pm 0.55^b$	$112.77 \pm 33.67^b$	$9.91 \pm 0.85^b$	$5.09 \pm 0.50^b$	$2.94 \pm 0.33^b$

\*\*\*Superscript with different letters within the column differs significantly at 95% confidence interval.

angle. The type of shell shape and color is influenced by both biotic and abiotic factors (Kovitvadhi et al. 2008). McDonald (2012) recorded different color morphs of adult and juvenile *Perna viridis*. According to the same author, some adult tropical mussel species, *P. viridis* in temperate Western Australia are bright green. The DMs found in Rutland Village in England are brown and yellow in color (Nature Spot 2020).

### 3.2 Water quality parameters

Key water quality parameters such as pH, Dissolved Oxygen (DO), Water Temperature (WT), transparency, and watercolor were recorded during the entire culture period. Independent T-test results revealed non-significant differences ( $p > 0.05$ ) among all the parameters studied. The mean pH, DO and water temperature range recorded was 8.04 - 8.18, 7.05-7.23 mg/L, and 16.44-25.7 °C, respectively. With monthly manuring and fertilization, a 20-60 cm water transparency level was observed with green-brown watercolor. When compared between treatments, the highest pH was recorded in the FWM concrete pond. Comparatively DM pond showed the highest DO and temperature. However, all the parameters recorded were within the optimal range that is ambient for the normal growth performance of native mussels.

In this study, pH ranged from 8.04-8.18, and the DO fluctuated from a minimum of 7.05 to a maximum of 7.23 mg/L. The water temperature ranged between 16.44°C and 25.70°C. Similar water quality ranges were recommended by Lajtner et al. (2004) for mussels' culture and then technically water quality does favor the farming of the mussels. According to the same authors, the favorable environmental variables for mussels in terms of pH, DO, and WT ranges from 7.63 – 8.69, 5.30 – 16.70 mg/L, and 5.80 – 22.0 °C respectively. The key water quality parameters observed fall within the recommended range that could have a positive impact on growth increment. Lewis

and Cerrato 1997 reported a positive correlation between growth and DO consumption from the research on growth uncoupling and the relationship between shell growth and metabolism in the soft-shell clam, *Mya arenaria*. The survival, growth, and condition of freshwater mussels were found ideal in municipal wastewater effluent that had pH, DO, and WT range of 8-8.2, 9.2-10.4mg/L, and 10.7-18°C respectively (Nobles and Zhang 2015). Another water temperature range of 16.18-20.67 °C and DO range of 9.45–11.88 mg/L were observed and reported by Azizi et al. (2020) during their scientific study on Mediterranean mussels, *Mytilus galloprovincialis* in Northern Morocco. The same authors reported that physicochemical parameters affect the growth indices and reproductive cycle. The above literature suggests that mussels in general are robust and rigid to thrive in the harsh and varied range of climatic conditions. During the culture period after the introduction of the mussels into the system, water quality improved significantly which indicates that mussels do filter detritus and debris in the water column. Thus, the presence of such a mechanism benefits pisciculture in automatic water quality regulations ensuring a safe haven for aquatic animals from murky and turbid rivers, especially during summers.

### 3.3. Host fish species

Both DM and FWM revealed successful interaction with the native warm water fish species such as barna baril (*Barilius barna*) (Hamilton 1822), zebrafish or zebra danio (*Danio rerio*) (Streisinger 1960) and ticto barb (*pethia ticto*) (Hamilton 1822), those found in Mau River. A few juvenile mussels were spotted during June month indicating a breakthrough in the unique reproductive cycle of the mussels. Therefore, the native fish species listed above fulfilled the requirement and function of host fish species in harboring glochidia for two to five weeks. Juveniles of DM weighing 0.19 to 0.96 g were detected in the concrete pond. The

presence of young mussels indicates the successful completion of the unique reproductive cycle of mussels. The culture of mussels along with native fish completes the unique cycle of harboring the glochidia in fish gills and fins after spawning that is compatible with mussels.

Mussels' right interaction with specific host fish is vital to ensure mussels' successful reproduction. It is critically important for the presence of host fish in relatively great abundance to complete an extraordinary reproductive cycle (Florida Fish and Wildlife Conservation Commission [FFWCC] 2019). As per the Conservation European Union Life (2014), Salmon and trout are two specific host fish for freshwater pearl mussels (*Margaritifera margaritifera*) found in Riparian Zone Rivers in Scotland. Salmon and trout play an instrumental role to complete the extraordinary lifecycle of pearl mussels. Largemouth bass, bluegill, and black-spotted topminnow are the potential host fish for Southern Fatmucket mussels found in Florida, USA.

### 3.4 Survival rate

During harvest, the population of mussels in the experiment was intently counted to ascertain the rearing mortality besides monthly mortality records. In total, 1085 numbers of mussels survived from a population of 1200 numbers. Specifically, 540 numbers (N = 600) of DM (90.00%), and then 545 numbers (N= 600) of FWM (90.83%) were harvested accounting for an overall 90.42% of survivorship in the concrete pond settings. A similar survival range for ridge mussels (*Amblema plicata*) had been reported by Nobles and Zhang (2015). Another author, O'Beirn et al. (1998) reported survival rate ranged between 26.85% and 85% for juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system which is comparatively lower than a concrete pond system, unlike this study. Therefore, comparatively the

culture of mussels in concrete ponds environment ensures a higher survival rate.

### 3.5 Onset of breeding

The juveniles of both DM and FWM were spotted during the month of June and July. When correlated with the water temperature, glochidia (young larvae) could be spawned during the month of March to April when the temperature rose above 15°C. However, an in-depth study featuring a detailed annual reproductive cycle of both mussels is recommended as a follow-up study with dedicated underwater cameras to capture accurate mussels' life cycles to ensure a complete understanding of natural propagation in Bhutanese soil.

## 4 CONCLUSION & RECOMMENDATION

DM to an extent is morphologically different from FWM, however, the latter is comparatively medium to large in size that is an important genetic resource for production. The robustness and rigidity of harsh management and environmental conditions encourage farmers to rear native species under the Bhutanese management systems, thereby, compensating for their low productivity. Both mussels are compatible with native fish species found in and around Gelephu for completing their unique reproductive cycle that requires host fishes. It is apparent that there is potential to improve native mussels for food production under the wings of aquaculture. Based on the current findings, it is recommended to initiate mussel farming to supplement aquaculture production since the propagation technology is adequately explored and documented. The aquaculture program has to give due emphasis and a concerted effort should be made to franchise proven mussel culture technology to derive maximum benefits from aquatic food diversity. Thus, there is a great scope in leveraging mussel production which will ensure additional income sources for fish farmers to boost the blue revolution and its economy.

## REFERENCES

- Azizi G, Layachi M, Akodad M, Baghour M, Skalli A and Moumen A. (2020). Growth weight and reproductive cycle in the mussel (*Mytilus galloprovincialis*) from Cala Iris Sea of Al Hoceima (Northern Morocco). *International Journal of Biosciences*. 16(6): 140-151.
- Bonel N and Lorda J. (2015). Growth and Body Weight Variability of the Invasive Mussel *Limnoperna fortunei* (Mytilidae) Across Habitat and Season, "Malacologia" 58, (1-2): 129-145. doi.org/10.4002/040.058.0202
- Borthagaray AI and Carranza A. (2007). Mussels as ecosystem engineers: Their contribution to species richness in a rocky littoral community. *Conservation EU*. (2014). Pearls in Peril. Conservation, EU Life, Freshwater pearl mussel, Pearls in Peril, Rivers. <https://freshwaterblog.net/2014/07/24/pearls-in-peril>. Accessed 13 September 2021.
- Department of Revenue and Customs. (2020). Bhutan Trade Statistics 2020. Department of Revenue and Customs, Ministry of Finance, Royal Government of Bhutan, Thimphu, Bhutan.
- FAO. (2016). Global per capita fish consumption rises above 20 kilograms a year. <http://www.fao.org/news/story/en/item/421871/icode>. Accessed on 19 August 2021.
- FFWCC. (2019). Freshwater mussel Host Fish Research: Researchers in Holt, Florida is studying freshwater mussel and host fish interactions to improve conservation efforts. <https://myfwc.com/research/freshwater/species-assessments/mollusks/host-fish>. Accessed on 3 October 2021.
- Kovitvadhi S, Kovitvadhi U, Sawangwong P, Trisaranuwatana P and Machado J. (2008). Morphometric relationship of weight and size of cultured freshwater pearl mussel, *Hyriopsis (Limnoscapha) myersiana*, under laboratory conditions and earthen pond phases. *Aquaculture International*. DOI 10.1007/s10499-008-9180-z.
- Lajtner J, Marusic Z, Klobucar GIV, Maguire I and Erben R. (2004). Comparative shell morphology of the zebra mussel, *Dreissena polymorpha* in the Drava River (Croatia). *Biologia*. 59(5):595-600.
- Lewis DE and Cerrato RM. (1997). Growth uncoupling and the relationship between shell growth and metabolism in the soft-shell clam, *Mya arenaria*. *Marine Ecology Progress Series*. 158: 177-189.
- National Biodiversity Centre. (2014). National Biodiversity Strategies and Action Plan 2014. National Biodiversity Centre. Yusipang, Thimphu, Bhutan.
- NSPWLR (2020). Duck Mussel - *Anodonta anatina*. <https://www.naturespot.org.uk/species/duck-mussel#tab2>. Accessed on 20 September 2021.
- Nobles T and Zhang Y. (2015). Survival, Growth, and Condition of Freshwater mussels: Effects of Municipal Wastewater Effluent. *PloS One*. 10(6).
- O'Beirn FX, Neves RJ, Steg MB. (1998). Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system. *American Malacological Bulletin*, 14(2): 165-171.
- RNR-SD. (2020). Annual Livestock Statistics 2020. Renewable Natural Resources Statistical Division, Directorate Services, Ministry of Agriculture and Forests, Thimphu, Bhutan.
- Yamane T. (1967). *Statistics, An Introductory Analysis*, 2<sup>nd</sup> Ed., New York: Harper and Row.