

## Chapter 10

# Nutrition

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### 1 Introduction

#### 1.1 Why nutrition?

By definition, a nutrient is a source of nourishment. We will define a nutrient as any absorbed or ingested molecule, organic or inorganic, required for *normal physiological functions* of an organism, such as survival, growth, maintenance, and reproduction.

Nutrition research should be structured so that daily dietary nutrient requirements are determined for sea urchins in different environments, culture conditions, and life-history stages. At this time, the daily nutritional requirement for any nutrient in most sea urchin species is not known. The inability to estimate daily dietary nutrient requirements is in part due to the differences in the approach used by various laboratories and deficiencies in experimental design (Watts et al., 2010). A goal of this paper is to summarize criteria and methods that should be considered in nutrition research for sea urchins and to review contributions that evaluate nutrients in relation to sea urchin survival, growth, reproduction, and health. Lawrence and Lawrence (2004) stated that a cost-effective, environmentally friendly commercial feed is an absolute requirement for sea urchin aquaculture. They emphasized that this feed would not only be an optimum nutritionally complete but also be optimal in terms of digestibility, incitants, stimulants, attractants, and physical characteristics (e.g., percent fines, hardness, leaching, size, shape, density, and stability). An optimum feed management program would consist of the number of feedings per day, amount of feed per day, percent of feed fed per day for each feeding, and the type of presentation of the feed.

#### 1.2 Nutrition vs dietetics

Most nutritional studies with sea urchins have been concerned with gonad and test production. Many studies have evaluated natural foods collected from the field in terms of survival, growth, and reproduction of a number of sea urchin species (Lawrence et al., Chapter 9). These studies are valuable in that they provide important information related to feeding ecology in sea urchins and can provide direction for nutrition research. However, evaluation of natural feeds does not provide information adequate to define daily quantitative nutrient requirements. This is because the nutrient content of natural foods cannot be completely determined. Natural foods can vary qualitatively and quantitatively in content with season, tissue, and/or location of collection. An exception would be a cultured natural food such as *Ulva* (Prato et al., 2018; Shpigel et al., 2018). Natural foods vary in physical and chemical characteristics, including size, shape, hardness, basic chemistry, and may contain varying levels of attractants, stimulants, and deterrents.

Few studies with sea urchins have investigated dietetics, the “practical application of diet in relation to health and disease” (The American Heritage Medical Dictionary, 2007). Dietetics research would allow us to make an inference as to potential requirements for nutrients for individual health but would not qualify as nutrition research per se.

### 1.3 Historical and contemporary approaches

An appropriate experimental approach to nutrition must consider the chemical and nutrient content of all feed ingredients in the diet and must be organized to evaluate appropriate outcomes. Standardized and valid methods must be used. These include a description of physical experimental conditions, desired physical and chemical characteristics of the feed, and proper feed management strategies.

Lawrence and Lawrence (2004) reviewed the early development of formulated diets. Following work with natural food, the next generation of feeds combined powdered, dry ingredients (e.g., soy meal, cornmeal, shrimp, fish meal) with a binder, usually agar or alginate, to form a moist pellet. These pellets usually had a high water content (>70% water) and, as such, had a short shelf life. Sea urchins readily consumed these feeds and reasonable growth rates were reported for several species (Klinger et al., 1994; Watts et al., 1998; Fabbrocini et al., 2012). These feeds exhibited several advantages. They could be prepared easily in any laboratory, could be processed to any size or shape desirable, had reasonably low leaching rates of dry matter, and could be prepared repeatedly with reasonable precision. However, moist pellets must be refrigerated, have a short shelf life of a few days, and are very expensive to prepare. Thus, moist feeds cannot be considered as feeds for a commercial aquaculture industry.

Dry feeds, presently being used in research and commercial aquaculture, contain <12% moisture and are produced by steam pelleting or extrusion cooking procedures. They can be produced in large quantities and have a long shelf life. Similar feeds have been developed for sea urchins (e.g., Hammer et al., 2004; Chang et al., 2005; Heflin et al., 2013).

## 2 Basic considerations in sea urchin nutrition research

Knowledge of the biology of a sea urchin increases the ability to investigate its dietary nutritional requirements (Watts et al., 2010). As with other aquatic species, sea urchins provide unique challenges to the study of nutrition. Basic life history characteristics vary among sea urchins (Lawrence and Bazhin, 1998) and a fundamental understanding of factors that affect feed acquisition and assimilation must consider this.

Prior history of the experimental animal must be reported. Abiotic and biotic factors (Table 10.1) must be considered and controlled at desired levels (Watts et al., 2010). In general, abiotic factors should be maintained as constant as possible during the experiment. Further, a growth rate of at least 80% of that observed in the field with above 90% survival should be obtained in the control group and used as a minimal criterion for dietary nutritional experiments.

Most of the factors in Table 10.1 have been considered in ecological studies or in studies related to feeding activity or consumption (Lawrence et al., Chapter 9), but not in the context of nutrition. Undoubtedly, all would affect daily dietary requirements and metabolic activity and, as a result, production.

One of the most challenging aspects in sea urchin nutrition research is the pattern, or lack thereof, of ingestion of food. Ingestion in most sea urchins is slow and irregular, making observations difficult. Unlike many aquatic and terrestrial species in culture, sea urchins will not always feed immediately with the introduction of food. Proffered food can remain in the water for some time prior to ingestion, resulting in substantial leaching. In addition to irregular ingestion, feed is

**TABLE 10.1** Abiotic and biotic factors potentially important in sea urchin nutrition research.

Abiotic factors	Biotic factors
Temperature	Life stage
Salinity	Size
Photoperiod	Sex
Light intensity	Conspecific interactions
Oxygen	Dominance
Water flow	Cannibalism
Water quality (e.g., NH <sub>3</sub> , NO <sub>2</sub> , NO <sub>3</sub> , pH, alkalinity)	Disease
Natural vs synthetic seawater	Symbioses

ingested by the activity of the teeth of Aristotle's lantern. Considerable food presumed ingested may be lost due to incomplete ingestion of shredded food. Further observations are needed to evaluate the biological and physical characteristics of food detection, acquisition, and satiation.

Another challenge in sea urchin nutrition is understanding the role of dissolved organic material (DOM). DOM may be very important in the early life history stages of sea urchin development (Manahan et al., 1983). It is doubtful that DOM will have a major role in sustaining juvenile and adult nutrition because of its low concentration. It could be important for the test and spines. External epidermal absorption of amino acids occurs in sea urchins (Bamford, 1982). External epidermal absorption of monosaccharides is probable. The contribution of DOM to total production is not known. Even a small percent contribution would be important. It is more likely that dissolved inorganic material will contribute to tissue production, particularly in terms of minerals such as calcium and magnesium as well as other minerals and metals. Heflin et al. (2012a) indicated that production efficiencies (dry feed consumed compared to dry tissue produced) in some individuals of *Lytechinus variegatus* exceeded 100% (up to ca. 180%), suggesting that in some conditions the mineral content of the water contributed significantly to test production.

### 3 Feed development—Dietary nutrient requirements

#### 3.1 Purified, semipurified, and practical ingredients

Feeds are classified based on ingredient and nutrient composition (Table 10.2). Some feeds are used to determine specific nutrient requirements of a species under defined experimental conditions. Others have commercial applications and are designed for large-scale production and use.

Purified, semipurified, and practical feeds are composed of dried purified and/or practical ingredients obtained from commercial sources. Purified ingredients are usually classified as a specific nutrient ingredient and are chemically defined. Most purified ingredients are largely a single nutrient or nutrient class and have been prepared from the chemical or mechanical separation of the parent ingredient. Practical ingredients usually represent crude foodstuffs that may be relatively unprocessed, dried, and ground to an appropriate particle size. These ingredients may vary significantly in composition (based on source), vendor, and season of collection. Their nutrient composition may be made of many different nutrients and/or not completely determined. Many practical ingredients contain known or unknown bioactive food compounds, representing nonnutrient molecules that can affect metabolism. Practical ingredients are used for commercial feeds to provide most of the required dietary nutrients for production. Nagai and Kaneko (1975) found that many practical ingredients commonly used in animal diets, but not found in the diets of natural populations (fish meal, shellfish meal, casein, gelatin, soybean meal, yellow corn, alfalfa, and yeast), can be ingested by *S. pulcherrimus*. When consumed most of these ingredients promoted growth. Several practical feed formulations have been tested in *Paracentrotus lividus*, with varying results and efficacy for somatic and gonad growth (Sartori and Gaion, 2016; Zupo et al., 2019). Purified ingredients with or without practical ingredients are used as needed to evaluate specific nutrient requirements while keeping other nutrients constant. Generally, survival and growth using practical feeds are greater than with semipurified feeds, which in turn are greater than with purified feeds.

**TABLE 10.2** Classification of feeds.

Type	Application	Content	Relative cost
Purified	Experimental feeds, used to evaluate macro- and micronutrient requirements	Purified, chemically defined ingredients, usually with defined lot numbers and specifications	High
Semipurified	Experimental feeds, used to evaluate macro- and some micronutrient requirements	Contain both purified and practical ingredients, not completely defined	Moderate
Practical	Commercial production feeds, produced in mass quantities for aquaculture operations, cannot be used to evaluate nutrient requirements	Contain practical ingredients, not chemically defined	Low

It is presumed that practical feeds have a higher probability to contain all the necessary nutrients. Practical and semi-purified feeds can be improved by the addition of nutrient requirements identified by studies with purified ingredients.

Only the nutrient being studied should vary in the diet to quantify the daily dietary requirement for this nutrient. This requires either purified or semipurified feeds. It is very difficult to determine dietary nutrient requirements using practical feeds since it is impossible to vary only a single nutrient using practical ingredients, each consisting of many different nutrients. However, practical feeds can be used to determine feed physical requirements, feed management requirements, physical system requirements, environmental requirements, and other production methods.

### 3.2 Physical feed characteristics

Physical characteristics include size, shape, texture, hardness, stability, and density of a feed pellet. Rounded or cylinder-like pellets are the most common shape produced by commercial extrusion or pelleting methods. [Klinger \(1982\)](#) reported the feeding rate of *L. variegatus* on large, block-like and terete agar-based prepared feed was greater than that of flat or prone feed. The optimum size of a pellet is also related to the amount of feed (ration) per feeding and the number of sea urchins in the culture system. If multiple sea urchins are in the system, the number of food pellets proffered per individual per feeding should be adjusted to limit competition. Since sea urchins are benthic, the feed pellet should sink. Effects of texture, hardness, and stability of feeds and their effect on ingestion have not been evaluated in any study. Soft pellets, such as those produced by agar-based feeds, are easily consumed but are not practical in commercial production. However, dry feeds that soften significantly after exposure to the water are usually avoided by the sea urchins ([Eddy et al., 2012](#)). Sea urchins should be able to eat hard feed pellets since sea urchin teeth can scrape and ingest hard surfaces including corals ([Klinger and Lawrence, 1985](#)).

### 3.3 Toxins in feed ingredients

Practical feeds may contain specific grains and/or cereals, a relatively cheap source of many essential nutrients. Several toxins found in some grain and cereal ingredients are produced by the plant and could reduce physiological function when consumed, resulting in reduced survival and/or growth rates. Examples are gossypyl and trypsin inhibitors in cottonseed and soybean meal, respectively. Further, during the production or storage of grains and cereals, specific fungi may grow, particularly under conditions of increased ingredient moisture levels usually above 12% and high temperatures, producing aflatoxins. Several toxins produced by fungi such as aflatoxins are very toxic to many aquatic and terrestrial species. Finally, natural food products such as seaweeds or kelp may contain secondary metabolites that negatively impact sea urchin performance and health ([Steinberg, 1984](#); [Bianco et al., 2010](#); [Lawrence et al., Chapter 9](#)). The response of small or large sea urchins to plant-produced toxins such as aflatoxins and other secondary metabolites have not been well characterized. They could affect metabolic outcomes.

### 3.4 Feed management

Feed management includes the amount of feed fed per day (feed ration), number of feedings per day, amount of feed per feeding, and time of day feed is proffered. As the quality of feed decreases, the feed nutrient density must be increased to obtain the same growth rate. For the determination of nutrient requirements, these criteria must be reported.

#### 3.4.1 Feed ration

Feed ration is the amount of feed proffered per individual or per group of individuals. Feed rations are usually proffered as a specific quantity [e.g., g individual<sup>-1</sup> or percent of sea urchin wet body weight or g (g wet body weight)<sup>-1</sup>]. These values are important and should be reported. An ad libitum ration greater or less than satiation can be used depending on the objective of the experiment. Since it is very difficult to determine the amount of feed consumed by sea urchins, proffering the feed at a ration of less than satiation (subsatiation) allows a more accurate determination of feed ingested and, thus, the daily quantitative dietary nutrient intake for the conditions of the experiment can be determined. In contrast, feed proffered ad libitum results in greater growth and estimates the maximum nutrient level in the diet required from maximum growth or some physiological parameter, again for the conditions of the experiment. Quantifying the amount of food consumed can be difficult with sea urchins, in which ingestion is irregular and unpredictable. Because of this, the amount of ingestion for several sequential days can be summed to increase the accuracy of the estimated ingestion rate.

### 3.4.2 Feeding rate

Feeding rate is the frequency of providing a feed ration. Feeding rate encompasses the number of times feed is proffered per unit time [e.g., g individual<sup>-1</sup> feeding<sup>-1</sup>, g wet body weight<sup>-1</sup> feeding<sup>-1</sup>, or percent of body weight day<sup>-1</sup> feeding<sup>-1</sup> (feeding is most often reported per day)]. Feed ration and feeding rates should not be confused with ingestion or rate of ingestion, which represent the amount of the feed consumed or consumed per unit time. Feeding rates are often determined by investigator workload considerations and costs, that is, the labor involved in conducting the experiment. However, inappropriate feeding rates will result in decreased growth rate or physiological function and can result in an inaccurate determination of the daily nutrient requirement of an optimum diet nutrient level. Nutrient leaching is an important consideration when using formulated feeds. Consequently, feeding rates must be adjusted to reduce the time that a feed is in the water before it is ingested. Lawrence et al. (2003) found that *L. variegatus* fed one ration per day had increased gonad production compared to those fed the same adjusted ration once every 2 or 4 days. Heflin and Watts (2015) found that *L. variegatus* fed daily had higher growth rates than those fed less frequently, regardless of ration size. Similarly, Wei et al. (2016) reported that *Strongylocentrotus intermedius* had greater growth and gonad production when fed daily for at least 12h, compared to those fed every other day or at 2-day intervals. Christiansen and Siikavuopio (2007) established a strong relationship between feed intake, gonad growth, and feed conversion ratio of large *Strongylocentrotus droebachiensis*. McCarron et al. (2009) found small *P. lividus* were more affected by intermittent feeding than large ones. These data suggest that sea urchins must feed daily to achieve maximal growth parameters.

### 3.4.3 Feeding time

Feeding time is the time of day the feed is proffered. There is no general consensus as to the appropriate time to feed sea urchins in nutrition studies. Most often feeding time in the laboratory is determined by investigator workload considerations. It is possible that feeding in the laboratory may be optimized at periods associated with changes in the light:dark cycle, but there are few data regarding optimal feeding times. Fuji (1967) first indicated that a change in light intensity caused a decrease in feeding and that low constant light conditions promoted constant feeding in *S. intermedius*. The specific time of feeding should be standardized and reported. The interaction of feeding time, feeding rate, and feed ration on ingestion rate and production need further study.

### 3.4.4 Feed ration at each feeding period

Under diel variations in light conditions, it may be desirable to vary the amount of feed proffered at different times as feeding activity may vary diurnally (Fuji, 1967). It is important to consider the amount of feed proffered relative to the time of feeding if the conditions of the experiment affect the amount of feed ingested. This has not been investigated in many sea urchins. Species may consume more of a ration at one time as compared to another time that same day. Thus, a ration would need to be modified to optimize ingestion at specific feeding times. There are several reports on diel variation in feeding in sea urchins in field populations, usually associated with the diel rhythm of predators (Lawrence, 1975).

### 3.4.5 Preexposure/sensitization

The nutritional and physiological history of a sea urchin at the beginning of a nutrition study may affect the outcome of requirement determinations for a specific nutrient. Sea urchins can store nutrient reserves in their gut and gonads (Lawrence and Lane, 1982; Bishop and Watts, 1992). As such, a specific nutrient being evaluated may be stored in these tissues and released, resulting in an inaccurate assessment of their potential role or function when they are limited in experimental feeds. For example, many minerals and fat-soluble vitamins can be stored in tissues and released. Consequently, a period of starvation may be required to limit the tissue availability of a specific nutrient in a subsequent experimental trial and/or the length of the experiment may need to be extended. For many micronutrients, it is important to determine the concentration of the nutrient under investigation within these organs prior to and at the end of nutrient requirement or restriction studies.

### 3.4.6 Group vs individual culture

Interactions of sea urchins in group culture may affect determination of daily nutrient requirements. To avoid interactions, sea urchins can be placed into containers and fed individually. Thus, individuals can serve as replicates within a treatment (not possible in group cultures unless individuals are marked). This approach is the most accurate for determination of

daily nutrient requirements and provides data on individual variability. For sea urchins held in group culture, broadcast feeding may be used, but individual ingestion rates cannot be measured. Group culture will provide relevant information on practical feed management in the presence of competition.

### 3.5 Outcome assessment

Outcome assessment (measurement or determination of a specific parameter or metric; for example, weight gain and gonad production) requires that specific outcomes be identified and evaluated for determination of specific nutrient requirements. The specific outcome being assessed will depend on the question or goal of the study. Several outcomes are used consistently and allow species comparisons.

#### 3.5.1 Ingestion

Quantitative daily requirements for specific nutrients cannot be accurately determined unless ingestion (feed intake and consumption) and efficiencies (digestibility) are measured. Ingestion can be reported as wet or dry matter ingested, or in terms of organic material, inorganic material, energy, or a specific nutrient. If sea urchins are fed *ad libitum*, the amount of feed not eaten must be determined if the quantification of the daily dietary requirement for one or more specific nutrients is the objective. This is difficult to determine accurately because of leaching, unstable feed, and inefficient eating habits of sea urchins.

#### 3.5.2 Digestibility

Ingestion of wet or dry food does not necessarily indicate that a specific nutrient is available biologically to the individual. Thus, digestibility (often referred to as absorption efficiency or availability) determinations are required for the evaluation of a specific nutrient or nutrient class. Digestibility refers to the amount of nutrient absorbed by the individual and is generally calculated as the amount of nutrient consumed minus the amount of nutrient retained in the feces. [Lawrence et al. \(2013\)](#) reviewed direct and indirect estimators of apparent dry matter digestibility (ADMD) and concluded that each had advantages and disadvantages. Direct determination (gravimetric collection of all feces for analysis) was an effective determinant of ADMD but time consuming. [Schlosser et al. \(2005\)](#) used acid-washed diatomaceous earth to indirectly estimate ADMD. Chromic oxide determinations were not accurate; since the gut of sea urchins processes food as a continuous flow, stirred-tank reactor, and chromic oxide will not accurately reflect digestibility in a single collection of feces (Lawrence et al., [Chapter 9](#)). Studies must also consider that various forms (organic vs inorganic), and sources (different vendors, batches or lots; practical vs purified) of specific nutrients or nutrient classes will vary in digestibility. Consequently, empirical determinations of specific organic and inorganic (mineral) nutrients are required to fully estimate nutrient digestibility. Digestibilities of natural and prepared feeds for various sea urchins are reviewed by Lawrence et al. ([Chapter 9](#)).

#### 3.5.3 Survival and growth

Survival and growth are common outcomes measured when evaluating daily nutrient requirements. Survival has limited value as an outcome for determination of daily nutrient requirements and is generally only a reflection of extreme limitation or toxicity of a specific nutrient. Organismal growth is the most common outcome used in determining nutrient requirements. Increase in dimension (horizontal diameter) is a measurement of growth but not of production, although it can be a quick estimate. Body wet weight is not an accurate measure of production because it includes the coelomic fluid, but does have comparative value. Weight gain, either wet or dry  $\text{g individual}^{-1}$ , provides a better estimation of growth or production (dry weight or organic weight gain) and can be expressed as an absolute weight (g) or as a percentage of the initial weight (percent increase). [Grosjean et al. \(1999\)](#) reported that immersion weight (mass determined in water) could accurately predict dry somatic weight in sea urchins fed a single diet. Specific growth rates (percent body weight gain per day, calculated as  $[(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{time (days)}] \times 100$ ) can be used to estimate organismal growth. It is most appropriately used when individuals are in the log phase of weight gain (usually as young juveniles) and not in the linear or asymptotic phase (small and large individuals). The functional consequence of specific nutrients may not be apparent in any outcome of growth but may be apparent in undefined physiological processes, including changes in disease resistance, reproductive state or in the production of viable offspring.



### 3.5.4 Effect of nutrients on organs

Because specific nutrients can affect nutrient allocation to specific tissues or organs, many studies measure wet, dry or organic material weight gain in four organs: test with spines, Aristotle's lantern, gut (esophagus, stomach, and intestine), and gonads. Organ weights can be compared directly among treatments or indirectly through the use of organ indices or ratios. Organ indices and ratios must be compared with caution, as incorrect interpretation can occur when allometric relationships are found. Differential nutrient allocation to calcified (test and lantern) vs noncalcified tissues (gut and gonad) is of major interest. Increases in calcified tissues are generally indicative of increased animal size and/or changes in shape, whereas noncalcified tissues reflect nutrient storage and/or allocation to gamete production. In particular, there is considerable interest in the gonads, as they are the primary nutrient storage organ in the sea urchin and are the organs of interest in aquaculture. Changes in the allocation of specific nutrients to specific organs will most likely have functional consequences to the individual. Despite widespread reports of organ growth in a variety of experimental and field conditions, we know very little about the basic physiology, biochemistry, and molecular biology of these organs in response to defined changes in specific nutrients.

### 3.5.5 Production, production efficiencies, and energetics

Production represents increases in dry or organic matter and is a common metric used in the evaluation of specific nutrients. In addition to production, the *rate* or *efficiency* at which production occurs can be a valuable metric for evaluating daily nutrient requirements. An accurate determination of the amount of feed ingested is required for production efficiency to be calculated. One of the most common metrics related to production is the food conversion ratio (FCR), defined as the dry matter of food proffered relative to wet weight gain. Although a relatively crude estimate of production rates, this metric has widespread and practical utility for many cultured species, aquatic and terrestrial, and reflects the ability of an organism to convert available food to biomass. Production efficiency based on rates of consumption (PEC), calculated as dry tissue produced relative to dry food consumed and expressed as a percentage (or organic tissue matter produced relative to organic food matter consumed), has greater utility for estimating the efficiency of converting food to biomass but requires accurate measurements of consumption (Watts et al., 2011). Most accurately, production efficiency can also be calculated based on the rate of absorption (PEA), calculated as dry tissue produced relative to dry food absorbed and expressed as a percentage (or organic tissue matter produced relative to organic food matter absorbed). In addition, production efficiencies in response to the ingestion or absorption of specific nutrients can be calculated. Production efficiencies have excellent utility in that they can be compared among various experiments and sea urchin species.

The energetic value of specific foodstuffs can affect ingestion and, consequently, production and production efficiency. *Lytechinus variegatus*, like many sea urchins, consumes food to apparent energy satiation (Taylor et al., 2015). It consumes higher amounts of low-energy feeds than high-energy feeds (Hammer et al., 2004; Taylor et al., 2015). Reduced ingestion based on high-energy food ingestion can significantly reduce the ingestion of specific nutrients, the limitation of which may also limit production. Alternatively, increased ingestion based on ingestion of low-energy feeds significantly increases the ingestion of specific nutrients, which may increase production. Taylor et al. (2015) reported that *L. variegatus* consumed more food and, consequently, more protein when fed a low carbohydrate feed (resulting in increased growth rates) than when fed a feed containing the same protein level but high levels of carbohydrates (higher carbohydrate energy).

Energy levels in relation to protein or other nutrients may be one of the most important metrics that affect production and production efficiencies (Powell et al., Chapter 4). The protein:energy ratio (P:E; sometimes reported as the energy:protein ratio) is an important metric for consideration when evaluating the proximate nutritional and energetic quality of feeds. The P:E is usually reported as mg protein  $\text{kJ}^{-1}$  or mg protein  $\text{kcal}^{-1}$ . Taylor et al. (2015) reported highest growth rates of juvenile *L. variegatus* when P:E ratios exceeded  $100 \text{ mg P kcal}^{-1}$ . As a nutrient, protein nitrogen can be an expensive component of sea urchin feeds. Protein can be consumed as an energy source, but it is desirable to spare dietary protein for maintenance and growth and use either lipids and/or carbohydrates as the primary energy source. Not all organic compounds are digestible or metabolizable, so caution must be used in interpreting the dietary contribution of these nutrients in various feeds. There is minimal information on protein and energy sources and values in sea urchin feeds.

Production efficiencies vary significantly within specific sea urchin populations. Heflin et al. (2013) found some individual sea urchins are more efficient in converting feed to biomass, and suggested these differences have a genetic basis. They hypothesized that variation in production efficiency evolved as an adaptive response to selective pressures related to food availability.

### 3.5.6 Functional or physiological metrics

The most common metrics used to evaluate daily nutrient requirements involve growth or growth-related indices, that is, metrics related to morphological parameters. However, many formulated feeds will contain adequate or excessive quantities of nutrients, and growth metrics may not vary significantly in response to changes in specific nutrients, particularly micronutrients. Although growth metrics may not be affected by some nutrients, there may be significant functional consequences in response to the presence or absence of these same nutrients. These effects would most likely be observed in specific physiological or metabolic pathways, reproductive success, or in the response to stress and disease. One of the most important outcomes for future evaluation is the immune response. Sea urchins have a complex innate immune system believed to involve various classes of coelomocytes (Tajima et al., 2007; Wang et al., 2013; Silva, Chapter 13). The extent to which these or other cells contribute to stress and disease tolerance and subsequent recovery is not known. However, we hypothesize that specific nutrients stimulate immunity, whereas limited or toxic levels of other nutrients reduce immunity. Nutrient effects on immunity have not been reported in sea urchins. Additional outcomes that measure the functional consequence of limited or toxic levels of specific nutrients need to be developed in sea urchins. These may include stress or disease tolerance and recovery as well as those mechanisms important in normal physiological function.

### 3.5.7 Role of gut flora

Lawrence et al. (Chapter 9) reviewed the occurrence and role of bacteria in the gut of sea urchins. There are few definitive reports that gut flora assist in digestion and absorption of nutrients, or whether specific nutrients affect populations of gut flora. Since the initial reports of Lasker and Giese (1954), molecular technologies have been developed that can assist in understanding the role of gut flora in digestion and absorption of nutrients. Using 16S rDNA sequence analysis, Nelson et al. (2010) found that *L. variegatus* held in the laboratory and fed a formulated diet contained a limited number of representative genera. They concluded that information on wild-type and captive-held sea urchin gut bacteria could help in the development of sea urchin aquaculture technologies. Using NexGen amplicon sequencing and downstream bioinformatics analysis, Hakim et al. (2016) reported a diverse microbial community in the gut of *L. variegatus* collected from field populations. Predictive metagenomics suggest that these microbial communities have an important role in carbohydrate, amino acid, and lipid metabolisms. Evaluation of microbial communities of *L. variegatus* held in culture and fed formulated diets further suggests that bacteria will be important in the processing of ingested nutrients (Hakim et al., 2015). Attributes of microbial metabolism in nutrient processing and energetics were further defined in the gut of *Strongylocentrotus purpuratus* (Hakim et al., 2019), indicating a very important role of the gut microbiome in sea urchin nutrition. In other aquatic species, gut flora can increase immune capacity, disease resistance, development and health of the intestine, and absorption and digestibility (Nayak, 2010). This needs investigation in sea urchins.

Orr et al. (2014) reported that *S. droebachiensis* readily consumed sablefish fecal waste, and showed production characteristics similar to sea urchins fed kelp. As solid fecal waste from most organisms contains numerous gut bacteria, it is likely that sea urchins consuming these waste pellets benefited both nutritionally and metabolically from the presence of bacteria. This was not considered in the study. Further information is needed on the role of bacteria in the gut and their response, or the consequence of their response, to specific dietary nutrients.

### 3.5.8 Molecular advances

The future of nutritional outcomes resides in the use of appropriate molecular tools, including genomics, transcriptomics, proteomics, and metabolomics. Zhang et al. (2013) indicate that growth rates of sea urchins are heritable. Thus, genetic evaluation for those sea urchins that show high weight gain, greater disease resistance, and quality roe production will aid in producing lines of sea urchins that can be cultured economically. Comparative evaluation of the transcriptome, proteome, and metabolome will allow us to evaluate metabolic processes associated with sea urchin production, allowing selection for value added and efficiency traits. This knowledge will be important for seed stock development in aquaculture operations.

## 4 Basic nutrient profiles

Many organisms share common metabolic pathways and, in many ways, will have similar nutrient requirements. Table 10.3 shows a synopsis of those nutrients that may be required in the diet. The quality and quantity of classes of nutrients (e.g., proteins, carbohydrates, fats and fatty acids, and carotenoids) differ with source (e.g., menhaden and soybean). Most of these potential nutrients have not been evaluated in sea urchins. Not only will these nutrients promote singular effects, there will be substantial interaction between and among many of these nutrients. These single and combinatorial effects also



**TABLE 10.3** Classes of nutrients suggested as being important in sea urchin nutrition.

Proximate nutrients	Proteins; essential and nonessential amino acids
	Carbohydrates (digestible); polysaccharides, oligosaccharides, monosaccharides
	Lipids; essential fatty acids, polyunsaturated fatty acids, highly unsaturated fatty acids, N3 and N6, carotenoids
	Ash (digestible and nondigestible minerals, e.g., diatomaceous earth)
	Fiber (insoluble and soluble, nondigestible carbohydrate)
Macrominerals	Calcium, magnesium, sodium, potassium, phosphate
Microminerals	Iron, zinc, manganese, copper, selenium, cobalt
Vitamins	Water soluble and fat soluble; ascorbic acid, tocopherols, calciferols, naphthoquinones, retinols, thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, biotin, folic acid, choline, inositol

will change depending on the age and physiological state of the sea urchin. They will also be affected by environmental factors. Some nutrients present in the diet in excessive levels will be inhibitory or toxic.

## 4.1 Protein

One of the most necessary nutrients required in a diet is protein, containing adequate levels of both essential (indispensable) and nonessential amino acids (dispensable). Protein is a vital macronutrient and is required by all eukaryotic organisms, including sea urchins, to maintain proper physiological functions (Powell et al., [Chapter 4](#)). It is one of the most expensive components in formulated diets. In sea urchins, protein most likely has a crucial role in many biological processes, including reproduction, early development, growth, and repair and maintenance of body tissues. Elevated dietary protein levels result in decreased feed intake ([Frantzis and Grémare, 1992](#); [Fernandez and Boudouresque, 1998](#); [McBride et al., 1998](#); [Meidel and Scheibling, 1999](#); [Agatsuma, 2000](#); [Fernandez and Boudouresque, 2000](#); [Hammer et al., 2004](#); [Daggett et al., 2005](#); [Hammer et al., 2006b](#)), increased somatic growth ([Fernandez, 1997](#); [Cook et al., 1998](#); [Fernandez and Boudouresque, 1998](#); [Fernandez and Pergent, 1998](#); [Meidel and Scheibling, 1999](#); [Agatsuma, 2000](#); [Akiyama et al., 2001](#); [Hammer et al., 2004](#); [Hammer et al., 2006b](#); [Hammer et al., 2012](#); [Heflin et al., 2012a,b](#); [Carboni et al., 2015](#); [Heflin et al., 2016a,b](#)), and increased gonad growth ([de Jong-Westman et al., 1995](#); [Fernandez, 1997](#); [Barker et al., 1998](#); [Cook et al., 1998](#); [Meidel and Scheibling, 1999](#); [Pearce et al., 2002a](#); [Hammer et al., 2004](#); [Schlosser et al., 2005](#); [Chang et al., 2005](#); [Hammer et al., 2006b](#); [Woods et al., 2008](#); [Hammer et al., 2012](#); [Heflin et al., 2012a, 2016a](#); [Powell et al., Chapter 4](#)).

Numerous studies have noted the importance of protein concentration in feeds on gonad production in sea urchins ([Phillips et al., 2010](#)). Several studies suggest that there is a maximal protein level, above which there is no further increase in the rate of somatic or gonadal growth ([McBride et al., 1998](#); [Kennedy et al., 2005](#); [Senaratna et al., 2005](#); [Hammer et al., 2006b, 2012](#); [Heflin et al., 2016a,b](#); [Powell et al., Chapter 4](#)) or even a decrease in weight gain ([Eddy et al., 2012](#)).

Exact dietary protein requirements for sea urchins have not been established. Differences in dietary protein requirements among species and age classes may be due to different experimental methods and diet ingredient levels and quality. [McBride et al. \(1998\)](#) reported highest production in *Strongylocentrotus franciscanus* at protein levels of 40% dry weight. *Pseudocentrotus depressus* fed a purified feed had the most test growth at protein levels between 20% and 50% dry weight and the highest feed efficiency at protein levels between 20% and 40% ([Akiyama et al., 2001](#)). [Hammer et al. \(2006b\)](#) reported optimal feed efficiency for adult *L. variegatus* at a protein level of 20% dry weight and recorded increased mortality with a 9% dry weight. Likewise, juvenile *L. variegatus* have a minimum protein requirement of 21% dry weight for optimal growth and survivorship ([Hammer et al., 2004](#)). [Pearce et al. \(2002b\)](#) found that both adult and juvenile *S. droebachiensis* require dietary protein around 20% dry weight. [Hammer et al. \(2004\)](#) also showed that increased intake of a lower protein diet increases gonad growth in *L. variegatus*. [Heflin et al. \(2016a\)](#) created predictive models that suggest that <30% protein (as fed) is required for maximal growth and gonad production in *L. variegatus*. Both the quantity and quality of proteins have not been evaluated for many sea urchin species. Glycine is the dominant free amino acid overall in *P. lividus*, *S. droebachiensis* ([Lee and Haard, 1982](#); [Liyana-Pathirana et al., 2002](#)), *Heliocidaris* (as *Anthocidaris*) *crassispina* ([Osako et al., 2007](#)), and *Evechinus chloroticus* ([Phillips et al., 2010](#)). [Phillips et al. \(2010\)](#) reported significant differences

in the concentration of individual free amino acids in testes and ovaries and diet of *E. chloroticus*. They suggested diet composition may influence the quantitative profile of free amino acids in the gonads. Similar results were reported by [Prato et al. \(2018\)](#), where free amino acids in the formulated diet could produce MYP (major yolk protein), a protein which is one of the primary storage proteins produced in the sea urchin ([Brooks and Wessel, 2002](#)). Identification of and requirement for dispensable and indispensable amino acids should be investigated.

Intake of excess dietary protein can be problematic. Metabolism of proteins is energetically costly, and protein yields less net energy in comparison to carbohydrates ([Powell et al., Chapter 4](#)). [Eddy et al. \(2012\)](#) reported that high protein levels (>23%) decreased weight gain and specific growth rate in small *S. droebachiensis*. High protein levels in manufactured feeds impart a bitter flavor to roe of *S. droebachiensis* ([Pearce et al., 2002b](#); [Kennedy et al., 2005](#); [Woods et al., 2008](#)). Authors have suggested that different amino acid contents of protein sources affect flavor of sea urchin roe differently ([Hoshikawa et al., 1998](#); [Pearce et al., 2002a,b](#); [Robinson et al., 2002](#); [Senaratna et al., 2005](#); [Woods et al., 2008](#)). [Phillips et al. \(2010\)](#) found a significant correlation between the concentration of valine in gonads and bitterness in *E. chloroticus*. Nitrogenous waste from protein is a pollutant in water systems ([Basuyaux and Mathieu, 1999](#)) and a major factor contributing to waste nitrogen, thus reducing growth and survival in commercial production systems. It is important that dietary protein requirements are satisfied but not exceeded when developing a sea urchin diet.

Studies examining the relationship between dietary protein and dietary energy requirements in sea urchins are needed. [Taylor et al. \(2015\)](#) concluded that protein consumption in relation to carbohydrate (energy) consumption was important in efficient use ingested nutrients, suggesting there is an optimal amount of energy required to efficiently use protein. [Heflin et al. \(2016a\)](#) used general linear modeling to predict that 18% dietary carbohydrate was more effective in promoting weight gain and gonad production than 12% carbohydrate, regardless of the amount of dietary protein. This study suggested that an appropriate protein:carbohydrate (energy) ratio should be considered when evaluating efficient nutrient utilization. [Heflin et al. \(2016b\)](#) used the geometric framework which involved allowing *L. variegatus* to choose between disparate diets based on nutrient content. This study found that *L. variegatus* have a tightly regulated intake target for dietary protein, but not dietary carbohydrate. Additional information concerning nutrient targets, including fat, will be extremely important in the formulation of a diet that provides adequate protein for optimal growth and production but spares protein as an energy source and reduces feed cost.

## 4.2 Carbohydrate

Organisms require diets that provide energy for maintenance, growth, and reproduction. Carbohydrates (saccharides) represent the most likely source of energy for sea urchins ([Powell et al., Chapter 4](#)). Additionally, saccharides are precursors for important nucleotides and as storage polymers. While amino acids derived from dietary proteins can be catabolized for cellular energy, the process is inefficient ([Powell et al., Chapter 4](#)) and energetically costly as compared to carbohydrate. Sea urchins may not be able to obtain dietary energy from lipids due to the low oxygen content in their tissues. However, soluble carbohydrates are easily processed by sea urchins. [Lawrence et al. \(Chapter 9\)](#) noted that many carbohydrates in the sea urchin gut oxidize complex polysaccharides into functional monosaccharides. Carbohydrates are stored by the nutritive phagocytes in the gonad ([Pearse and Cameron, 1991](#); [Powell et al., Chapter 4](#); [Hammer et al., 2006a](#)). Stored carbohydrates may affect taste ([Pearce et al., 2002a,b](#); [Unuma, 2002](#)) and/or provide energy for gametogenesis ([Pearse and Cameron, 1991](#); [Powell et al., Chapter 4](#)). Carbohydrates are a much more efficient energy source than protein in sea urchins ([Powell et al., Chapter 4](#)). Evaluating the direct effects of carbohydrates may be confounded by their direct and indirect interaction (utilization) with other nutrients, particularly protein, and the level of nonmetabolizable components of the diets such as total ash and fiber.

The direct effect of carbohydrates (quantity or quality) on sea urchins has received little attention, despite their potential role in promoting normal physiological function. [Eddy et al. \(2012\)](#) and [Heflin et al. \(2012a\)](#) reported that dietary carbohydrates (starch) ranging from 21% to 39% dry weight of the diet did not affect weight gain or organ production in *S. droebachiensis* or *L. variegatus*, respectively. In studies where dietary carbohydrates were altered, both [Taylor \(2006\)](#) and [Hammer et al. \(2006a\)](#) suggest that *L. variegatus* may adjust feed intake to satisfy an energy requirement. Juvenile *L. variegatus* fed a lower energy feed (less carbohydrate) consumed significantly more feed and, as a consequence, consumed significantly more protein than individuals receiving feed with an equivalent protein level but a higher carbohydrate level ([Taylor et al., 2015](#)). A similar trend was seen in adult *L. variegatus*. [Hammer et al. \(2012\)](#) observed an inverse relationship between intake and carbohydrate level of the diet regardless of decreases in protein intake. [Heflin et al. \(2016b\)](#) found that daily dietary carbohydrate intake could vary significantly, suggesting that carbohydrate intake was not tightly regulated. [Lawrence et al. \(2011\)](#) saw similar feed intake trends in *S. intermedius* fed two isocaloric feeds with different levels of protein and carbohydrates. However, variations in protein intake did not have a significant effect on growth or production.

Sea urchins have a low respiration rate (Lawrence and Lane, 1982). They are relatively sedentary, suggesting that their energy requirement should be low. Therefore, it is reasonable to expect that a nutritionally complete sea urchin diet would have a high protein:energy (carbohydrate) ratio. The study by Taylor (2006) supports this assumption. Juvenile *L. variegatus* fed formulated feed containing 104–112 mg protein kcal<sup>-1</sup> (low carbohydrate) had higher somatic growth than individuals receiving feed containing 82 mg protein kcal<sup>-1</sup> (high carbohydrate). Hammer et al. (2012) and Heflin et al. (2012a) obtained similar results in adult *L. variegatus* fed diets with varying protein:energy (carbohydrate) ratios. *S. intermedius* fed isocaloric diets with varying protein:carbohydrate ratios had higher gonad organic matter production with a higher protein:energy ratio but showed no difference in wet weight or in wet test, lantern gut, or gonad index (Lawrence et al., 2011). This suggests the lower protein:energy ratio diets were adequate for growth and production. In contrast, *L. variegatus* receiving adequate protein but low levels of dietary carbohydrates also had decreased growth and production (Heflin et al., 2016a). This suggests that the dietary protein is used as an additional energy source when carbohydrate levels are limiting (Schlosser et al., 2005; Hammer et al., 2006a). Decreased survival has also been observed in *S. droebachiensis* when dietary protein levels are inadequate to compensate for low carbohydrate levels (de Jong-Westman et al., 1995).

The contribution of carbohydrate (quality or quantity) needed to satisfy energy requirements for processes unrelated to weight gain (e.g., gamete production and maintenance) has not been determined. For juvenile sea urchins, the carbohydrate content of a diet should be sufficient to provide the sea urchin with enough metabolic energy to promote somatic growth but not be in excess that results in precocious production of nutritive phagocytes in the gonads (obesity).

### 4.3 Lipids

Lipids represent a diverse class of organic molecules that includes triacylglycerols, phospholipids, sterols, waxes, carotenoids, and free fatty acids. The nutritional requirement for lipids depends on an organism's ability to synthesize lipids or acquire various lipid molecules through the diet. Stage of growth and development must be considered since periods of rapid growth generally require more lipids in the diet as de novo synthetic processes become limiting. Understanding the functional role of lipid is needed [e.g., energy source, essential fatty acid (EFA) source, structural lipids, hormone precursor] and will be important in determining the combination of lipids necessary for normal physiological function.

In marine invertebrates, triacylglycerols (neutral lipids) can serve as an important source of essential fatty acids and energy (Giese, 1966; D'Abramo, 1997). Sea urchins primarily store lipids in the gut and gonads, and lipids can also be found in the coelomic fluid (Giese, 1966; Lawrence et al., 1966; Bishop and Watts, 1992). Neutral lipid levels decrease in the gut of starved *Strongylocentrotus purpuratus* (Lawrence et al., 1966; reviewed by Giese, 1966), suggesting that lipids are an energy store for sea urchins. The dietary requirement for neutral lipids has been evaluated for small *S. droebachiensis* (Castell et al., 2004; Kennedy et al., 2007a; González-Durán et al., 2008), small *L. variegatus* (Gibbs et al., 2015), and for adult *L. variegatus* (Hammer et al., 2010; Gibbs et al., 2013) by using marine and/or plant oils in the formulation. While the dietary lipid and essential fatty acid requirements of sea urchins have yet to be determined, high levels of neutral lipids in the diet negatively affect growth (Kennedy et al., 2007a; Hammer et al., 2010; Gibbs et al., 2015). This may be due to nonoptimal energy levels and/or fatty acid profiles for a particular species, life stage, or environmental condition.

The inclusion of polar lipids (phospholipids) in the diet can provide a significant complement of nutrients in the form of fatty acids, choline, inositol, ethanolamine, phosphorus, or related components. They also function as an emulsifier and improve feed digestion and nutrient translocation in marine invertebrates (Teshima, 1997; Gong et al., 2000; Tocher et al., 2008). When fed a diet containing 5% dry weight corn + linseed + menhaden oils (1:1:1) with 3% dry weight soy lecithin, small mature *S. droebachiensis* had improved growth and lipid deposition in gonadal tissues (González-Durán et al., 2008). Gibbs et al. (2010) found that inclusion of 4% dry weight soy lecithin in a formulated diet supported maximal gonad production in adult *L. variegatus*. High dietary lipid content increased the gonad index in *P. lividus* but did not affect overall lipid content in the gonad (Carboni et al., 2015). Growth in small *L. variegatus* decreased at soy lecithin levels above 6.4% dry weight. Inclusion of 1% dry weight soy lecithin in the diet was sufficient to produce maximal weight gain and gonad production (Gibbs et al., 2009). In this study, excess phospholipids in the diets was presumably stored as triglycerides in the gut and gonads. Inclusion of 2% dry weight soy lecithin in the diet of juvenile *L. variegatus* decreased weight gain, indicating the requirement for dietary phospholipids is minimal in individuals prior to gonadal growth. It also suggests age-specific requirements for dietary phospholipid. The ability to synthesize phospholipids de novo has been reported for sea urchins (Ellington, 1982). However, a specific dietary requirement for phospholipids in juvenile and adult sea urchins remains unclear.

For marine invertebrates, cholesterol serves as a structural element in cell membranes and a precursor for steroid hormone production (Giese, 1966; Teshima, 1997). Optimum levels of dietary cholesterol can range from 0.12% to 2% dry weight for crustaceans (Teshima, 1997). In juvenile shrimp, *Litopenaeus vannamei*, dietary levels of phospholipids

of 1.2% or 3% with supplemental cholesterol of 0.2% resulted in optimal growth (Gong et al., 2000). Sea urchins are capable of synthesizing cholesterol *de novo* (Voogt, 1982). However, the degree to which dietary cholesterol is required is not known. Gibbs et al. (2010) reported the inclusion of up to 0.6% dry weight cholesterol in a semipurified diet did not affect organismal or gonad production. However, endogenous cholesterol in practical ingredients may have satisfied any requirement or cholesterol could have been synthesized from precursors (Voogt, 1972; Smith and Goad, 1974). Nutritional work investigating dietary cholesterol requirements (particularly in using purified diets) in sea urchins is needed.

While the form of dietary lipid can be important for growth performance, the source or quality of that lipid must also be considered. Essential fatty acids (EFA) composed of the linoleic ( $n - 6$ ) and linolenic ( $n - 3$ ) families of polyunsaturated fatty acids (PUFAs), and highly unsaturated fatty acids (HUFAs), must be supplied in the diet. Generally, the nutritive value of  $n - 3$  PUFAs is higher for marine organisms than the  $n - 6$  PUFAs (D'Abramo, 1997). Echinoderms are capable of *de novo* synthesis of neutral lipids. However, their ability to synthesize  $n - 3$  and  $n - 6$  18C PUFAs is not fully understood (Ellington, 1982). *Strongylocentrotus droebachiensis* fed diets deficient in EFA had poor growth and an accumulation of 20:3 $n - 9$  in the test, a potential indicator of EFA deficiency (González-Durán et al., 2008). Sea urchins can synthesize 20:4 $n - 6$  (arachidonic acid) and 20:5 $n - 3$  (eicosapentaenoic acid, EPA) from 18C PUFAs and 20 and 22C non-methylene-interrupted dienoic fatty acids (NMIDs) (Bell et al., 2001; Castell et al., 2004; Liu et al., 2007a,b; González-Durán et al., 2008). Prato et al. (2018) suggested *P. lividus* could synthesize significant quantities of PUFA from a formulated diet, the fatty acid quality of which was dependent on the quality of the diet. However, an overall reduction in EPA and DHA was observed in gonad tissues. Zuo et al. (2018) reported that supplementing a diet with 1% arachidonic acid resulted in an increase in wet weight and gonad index for *S. intermedius*, supporting the value of  $n - 6$  fatty acids in sea urchin growth and reproduction.

Fatty acid composition of the diet is reflected in sea urchin tissues (Liyana-Pathirana et al., 2002; Castell et al., 2004; Liu et al., 2007a,b; González-Durán et al., 2008; Prato et al., 2018). The diet should contain appropriate ratios of  $n - 6$ : $n - 3$ , saturated:unsaturated, and PUFAs:HUFAs to satisfy nutritional requirements. Because marine oils (menhaden and other fish oils) that supply high nutritive value  $n - 3$  HUFAs for marine organisms (D'Abramo, 1997) can be very costly, lipid replacements from plant sources are commonly used in formulated feeds. Plant oils typically have a higher 18:2 $n - 6$  (linoleic acid) content and, as a result, a higher ratio of  $n - 6$ : $n - 3$  fatty acids. Although unknown in invertebrates, a high ratio of  $n - 6$ : $n - 3$  fatty acids has been suggested to lead to an inflammatory response and a number of chronic diseases in humans (De Caterina and Basta, 2001; Simopoulos, 2006). High levels of 18:2 $n - 6$  in the diet of *S. droebachiensis* resulted in high levels of 20:4 $n - 6$  in tissues, while dietary 18:3 $n - 3$  resulted in high 20:5 $n - 3$  fatty acids in tissues (Castell et al., 2004). Since 20:4 $n - 6$  fatty acids are precursors for eicosanoid production, responsible for inflammatory responses (Funk, 2001), accumulations of 20:4 $n - 6$  in sea urchin tissues may cause a similar response and may decrease growth. Dietary fatty acid ratios for sea urchin feeds need to be evaluated. Both quantitative and qualitative assessment will be required.

Carotenoids represent an important class of lipids whose biological effects may be nutritional as well as physiological. Suckling et al. (Chapter 11) describe both nutritional (precursor for nutrients such as vitamin A) and physiological (photo-protection, reproduction) roles for carotenoids in sea urchins. When different carotenoids are included in the diets, biological effects concerning reproduction processes as well as structural changes can be observed. This suggests an important role for various classes of carotenoids in the diets of sea urchins. Carboni et al. (2015) suggested fecundity of *P. lividus* was enhanced when xanthophyll was placed in a formulated diet. De Jong-Westman et al. (1995) reported the inclusion of  $\beta$ -carotene in prepared diets significantly increased gonad growth in *S. droebachiensis*. In contrast, Pearce et al. (2003) stated it was not clear why they found prepared diets with  $\beta$ -carotene had significantly lower gonad production in *S. droebachiensis* than diets without the pigment.

#### 4.4 Fiber

The role of fiber as an essential nutrient has not been determined in sea urchins. Fibers are generally classified as soluble or insoluble. Both have different functions in both herbivores and carnivores. Despite the fact that sea urchins often consume natural biota (kelps and algae) that can contain high levels of insoluble fiber (cellulose), these compounds are essentially not digested (Lawrence, 1982; Lawrence et al., Chapter 9) but maybe important for normal gut function and gut passage time for food. Inclusions of fiber (alpha-cellulose) ranging from 1.2% to 5% dry matter into formulated feeds did not affect growth or production in *L. variegatus* (S.A. Watts, unpublished). The contribution of fiber to sea urchin growth or health has not been examined.

#### 4.5 Minerals

Minerals, usually in elemental forms, are essential for normal physiological function for all organisms. For many plant and animal species, minerals are a minor component of the total body composition. However, minerals can comprise a



substantial percentage of the dry matter content of sea urchins, primarily in the test, spines, and Aristotle's lantern. These organs require significant quantities of the macrominerals calcium and magnesium, which form the calcium–magnesium carbonate skeleton (Ebert, Chapter 7). These and other minerals can be absorbed directly from the seawater and/or obtained from the diet. Dietary requirements for any mineral have not been determined. Calcium, the most abundant mineral found in sea urchins, is readily absorbed by the gut (Kaneko et al., 1981). Powell et al. (2010) reported increased weight gain and diameters in small *L. variegatus* fed calcium-supplemented formulated diets (dose dependent), suggesting a dietary calcium requirement of at least 4.7% dry weight. In contrast, elevated magnesium levels (0.4% dry weight) inhibited weight gain, suggesting the ratio of calcium–magnesium may be important. Other macrominerals (phosphorus, sulfur, sodium, potassium, chloride) should be required in lesser quantities, but dietary requirements are not known. Powell et al. (2010) did not find a requirement for dietary phosphorus in small *L. variegatus*. This is not surprising as hydroxyapatite (containing phosphorus) is found in vertebrate bone. Kennedy et al. (2007b) indicated that the addition of commercial mineral premixes (containing macro- and micromineral salts and conjugates) to a formulated diet enhanced growth of juvenile *S. droebachiensis*. Growth was further enhanced by the addition of carotenoids. Jones et al. (2010b) did not find a dietary requirement for selenium in *L. variegatus* but did report pathologies at 2.4 ppm similar to that reported for other aquatic animals, suggesting toxicity at high concentrations. Jones et al. (2010a) reported growth (increased diameter) associated with dietary zinc, suggesting a dietary requirement of at least 0.15% dry weight, but did not find growth effects with iron or manganese in small *L. variegatus*.

## 4.6 Vitamins

Vitamins represent a class of small organic compounds that are essential in many metabolic pathways. They are characterized by their activity and are usually effective in very small amounts. They cannot be synthesized de novo by the animal. Historically, vitamins are classified by their solubility in either aqueous or nonpolar solvents (water and fat-soluble are the classical terms used to describe their chemical affinity). Alternatively, they are classified based on their related (family) activity [e.g., vitamin D, vitamin A, and vitamin B all have multiple forms (vitamers) within their family]. Dietary vitamin requirements have received little attention in sea urchins. Vitamin content has been measured in *Paracentrotus lividus* gonads (de Quirós et al., 2001a,b) and provitamin A carotenoids and vitamin C and E in *P. depressus* (Kawakami et al., 1998). Shimada et al. (1993) reported a family of vitamin B complex vitamins in the gonads of *Hemicentrotus pulcherrimus*, but no function was noted. Lukyanova and Khotimchenko (1995) reported vitamin E in ovaries and testes of *S. intermedius*. The gut of *S. droebachiensis* contained folate, niacin, pantothenic acid, thiamin, riboflavin, retinol, and alpha-tocopherol (Mamelona et al., 2010), further suggesting a functional role in that organ. Carotenoids, as vitamin precursors, are discussed by Suckling et al. (Chapter 11). The presence of multiple vitamins in sea urchin tissues suggests a functional role in physiological processes.

Jones et al. (2012) found the addition of a commercial vitamin premix to formulated diets enhanced the growth of small *L. variegatus*. They indicated growth might be inhibited at high concentrations, suggesting possible inhibition by one or more vitamins. It was not possible to determine specific vitamin requirements, but the study suggests one or more vitamins are required in the diet to promote weight gain. Hobbs and Pennock (1977) detected the presence of an isomerizing system in sea urchins that were capable of forming precholecalciferol from vitamin D and vice versa in *Psammechinus miliaris*. This suggests a possible role for vitamin D. Tsushima et al. (1997) suggested that dietary vitamin E might affect ovulation in *P. depressus*, but other effects on development were not conclusive.

## 4.7 Feed additives

Feed additives represent various classes of molecules, compounds, or organisms that promote ingestion, absorption, assimilation of nutrients, growth, and health. They affect physiological processes, such as immune function, stress resistance, and reproduction. Feed additives include feeding attractants, immunostimulants, prebiotics, probiotics, acidifiers, essential oils, or other inclusions. These additives are usually not required nutrients.

Feed attractants enhance the ingestion of feeds. Natural feed attractants have been characterized in many sea urchin species, and their efficacy in feeds has been reviewed by Lawrence et al. (Chapter 9). Sea urchins can show feeding preferences due to the presence of attractants, resulting in increased feed intake. This would be particularly important in formulated diets, where some ingredients (nutrients) are water soluble or are expensive, and rapid ingestion is highly desirable. Immunostimulants have received minimal attention. Beta-glucans are presumed immunostimulants in most aquatic organisms (Soltanian et al., 2009). Laminarin increased embryo survival in *S. intermedius*, but the exposure was waterborne and not dietary (Kiseleva et al., 2008). Vitamins and minerals often promote an immune function, but these are not considered immunostimulants as they function primarily to assist normal immune reactions. Although an adaptive immune

response is not seen in the Echinodermata (Silva, [Chapter 13](#)), stimulation of phagocytic activity by coelomocytes (innate immune response) may be improved by immunostimulants.

Prebiotics, such as acidifiers, essential oils, probiotics, and antibiotics are all feed additives that optimize the gut flora of fish and shrimp, enhance the immune response, increase stress resistance, promote animal and gut health, and increase digestion and nutrient availability ([Anuta et al., 2011](#)). Molecules and compounds stimulating a positive response of gut microflora and probiotics (gut microflora enhancing a biological response) have not been evaluated as feed additives in sea urchins.

## 5 Basic applications of feed development

Aquaculture of sea urchins for roe has been the primary stimulus for nutritional studies. However, aquaculture has an important role in providing sea urchins for biomedical research. It provides a predictable supply of standardized individuals of known nutritional condition for experimental studies. Successful land aquaculture for these purposes requires the development of formulated feeds. Of critical importance is the determination of dietary nutrient requirements, which are required for the development of a cost-effective commercial dry or semimoist feed. Dry or semimoist formulated feeds are an absolute essential requirement for successful and sustainable sea urchin aquaculture because they (1) are nutritionally more complete and consistent in composition and nutrient density, less expensive, less polluting, and less labor intensive than natural foods such as algae, (2) represent the largest variable cost and thus mandate a cost effective, least-cost formulation for the desired return on investment, (3) are required for commercial production in terms of maximum growth and survival, (4) serve as a means for incorporating dietary feed additives for growth enhancement, immune response, etc., and (5) can represent the major source of pollution to the environment ([Lawrence and Lawrence, 2004](#)).

## 6 Summary

Limited knowledge is available concerning the dietary nutrient requirements and optimum nutrient levels in the diet of sea urchins. However, even with this limited knowledge adequate purified and semipurified diets have been formulated for research and practical diets formulated for the production of roe for human consumption.

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