



J. Plankton Res. (2022) 1–17. <https://doi.org/10.1093/plankt/fbac064>

ORIGINAL ARTICLE

Genetic variability, biomass parameters, elemental composition and energy content of the non-indigenous hydromedusa *Craspedacusta sowerbii* in North America

FLORIAN LÜSKOW ^{1,2,*}, MAARTEN BOERSMA ^{3,4}, PABLO J. LÓPEZ-GONZÁLEZ ⁵ AND EVGENY A. PAKHOMOV ^{1,2}

¹DEPARTMENT OF EARTH, OCEAN AND ATMOSPHERIC SCIENCES, UNIVERSITY OF BRITISH COLUMBIA, 2039–2207 MAIN MALL, VANCOUVER, BRITISH COLUMBIA V6T 1Z4, CANADA, ²INSTITUTE FOR THE OCEANS AND FISHERIES, UNIVERSITY OF BRITISH COLUMBIA, 2202 MAIN MALL, VANCOUVER, BRITISH COLUMBIA V6T 1Z4, CANADA, ³ALFRED-WEGENER-INSTITUT HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG, BIOLOGISCHE ANSTALT HELGOLAND, KURPROMENADE, HELGOLAND 27483, GERMANY, ⁴FACHBEREICH 2 BIOLOGIE/CHEMIE, UNIVERSITÄT BREMEN, LOEBENER STRASSE, BREMEN 28334, GERMANY AND ⁵BIODIVERSIDAD Y ECOLOGÍA ACUÁTICA, DEPARTAMENTO DE ZOOLOGÍA, UNIVERSIDAD DE SEVILLA, AVDA. REINA MERCEDES 6, SEVILLA 41012, SPAIN

*CORRESPONDING AUTHOR: flueskow@eoas.ubc.ca

Received June 28, 2022; editorial decision October 14, 2022; accepted October 14, 2022

Corresponding editor: Beatrix E. Beisner

The freshwater hydrozoan *Craspedacusta sowerbii* was introduced to habitats around the world and they spread into the surrounding water bodies thereafter. Despite an increase in reported observations, little is known about its genetics, physiology and ecology. The present study examined the organic content, elemental composition and energy content of jellyfish collected in Canada and the USA and compared these metrics with their marine counterparts and other co-occurring zooplankton. Specimens from the sampled lakes were sexed and molecularly characterized. Molecular, stoichiometric and energetic information is critical in determining distribution pathways and trophic connections. Molecular analyses showed two closely related sequences in North America, one shared by the Canadian and Japanese specimens, while another one is widely present across the Americas. Organic and all elemental contents were higher compared to marine jellyfish species. Estimated energy content of *C. sowerbii* was 20–27 kJ g DW⁻¹, which is 2-fold higher than for marine hydrozoans and compared well with cladocerans, copepods and rotifers. Estimated C/P values indicated that cladocerans and copepods are suitable prey for *C. sowerbii* and that jellyfish may compete with juvenile

fish to fulfill similar nutrient demands. Freshwater jellyfish were stoichiometrically and energetically different from marine hydrozoans, indicating higher importance in freshwater food webs than previously thought.

KEYWORDS: Cnidaria; gelatinous zooplankton; intraspecific variability; invasive species; non-indigenous

INTRODUCTION

Gelatinous and soft-bodied zooplankton (GZ) are ubiquitous components of almost all aquatic food webs, yet their ecological roles are often unclear (Hays *et al.*, 2018), and, as a result, often ignored in food web models (Pauly *et al.*, 2009; Lamb *et al.*, 2019). In the absence of species-specific energy and elemental composition data, GZ taxa (but rarely their benthic life cycle stages) are often pooled together. Neglecting functional diversity, however, may lead to erroneous interpretations of the GZ roles in food webs (Lüskow *et al.*, 2021a). GZ include diverse taxonomic and functional groups (i.e. medusae, siphonophores, ctenophores, pelagic tunicates and pelagic gastropods) but do have some common traits, i.e. transparent tissues/bodies, high body water contents facilitating near neutral buoyancy, fast growth, generally large size of adults compared to other zooplankton, and often, a lack of hard structures making them fragile, difficult to sample and nearly invisible in traditional diet studies (Madin and Harbison, 2001). A growing body of literature, however, provides information that GZ in the marine realm are consumed by a multitude of predators ranging from scavenging benthos organisms to marine mammals (e.g. Hays *et al.*, 2018) despite their generally low energy contents (Lüskow *et al.*, 2021a). Such feeding interactions are considerably less studied for freshwater GZ. The nutritional value of GZ depends not only on their energy content but also on their biochemical composition. In ecological stoichiometry, ratios between elements, such as the carbon-to-phosphorus ratio (C/P value), are often used to express the value of a food item, whereby a high C/P value typically describes food of low quality, as the P may be limiting (van de Waal *et al.*, 2010). The ratio at which P becomes limiting is species-specific (threshold elemental ratio; Khattak *et al.*, 2018) and may even change with development and activity. Unfortunately, such food quality data are extremely rare for GZ organisms (Lüskow *et al.*, 2022), which makes generalizations of their trophic roles speculative. Especially for smaller, cryptic GZ species, such as members of the Olindiidae family, there is an urgent need for more data (Pauly *et al.*, 2009; Lamb *et al.*, 2019).

Olindiidae (Cnidaria, Hydrozoa) is a small family of Limnomedusae, containing <50 species in 16 genera (Daly *et al.*, 2007; Collins *et al.*, 2008). Most olindiid species demonstrate a metagenetic life cycle, i.e. they alternate

between an asexually reproducing polyp stage (in addition to frustules and podocysts) and a sexually reproducing medusa stage (Jankowski, 2021). Despite its small size, the family Olindiidae is remarkable with respect to inhabited environments, as its members are found in freshwater (e.g. *Craspedacusta* and *Limnocnida*), brackish (e.g. *Maeotias*) and marine (e.g. *Eperetmus* and *Gonionemus*) habitats. This habitat diversity is unique among cnidarian taxa. Several species in this family (e.g. *Craspedacusta sowerbii*, *Gonionemus vertens* and *Maeotias marginata*) even manage to maintain self-sustaining populations in non-indigenous habitats worldwide (Väinölä and Oulasvirta, 2001; Marchessaux *et al.*, 2017, 2021; Lüskow *et al.*, 2021b). Their dispersion potential likely results from a wide abiotic tolerance and diverse reproduction traits. However, despite increasing interest, knowledge of their biology, ecology and physiology remains limited, with the medusa stage (= jellyfish) better studied than the cryptic polyp stage (Lüskow *et al.*, 2021b; Salonen *et al.*, 2012). Biomass parameter and stoichiometric changes related to ontogeny have not yet been reported for any olindiid species and medusa growth, as well as development, and remain only sparsely investigated (e.g. Larson, 1986; Jankowski, 2000; Salonen *et al.*, 2012), which motivated this study that focused on a well-known olindiid species—the freshwater hydrozoan *C. sowerbii*.

Craspedacusta populations in non-indigenous habitats are often unisexual (e.g. Lüskow *et al.*, 2021b), which may be traced back to the introduction events of single or a few polyps or podocysts of one sex. The genetic diversity in these sites is therefore expected to be very low, with some populations built entirely of identical clones. Medusa budding under favorable conditions still happens in these sites, but sexual reproduction and thus recombination of genes is absent. However, specimens of different genetic lineages were reported previously on relatively small scales (Oualid *et al.*, 2019; Mörpurgo *et al.*, 2021; Peterson *et al.*, 2022). Despite global spreading, the genetic variability among and within populations has rarely been studied. This is particularly true for invaded sites of North America. While in some regions of the USA, for instance, VA, *C. sowerbii* has been reported for almost a century (e.g. Hamaker and Milne, 1937), the hydrozoan was only relatively recently introduced to regions at its northernmost distribution boundary, such as in British Columbia, Canada (Marchessaux *et al.*, 2021; Lüskow *et al.*, 2021b). Knowledge about the genetic variability

and thus the ability to adapt to changing environments is especially important at distribution boundaries not only in North America but also in Europe, Asia and South America.

Similar to the limited understanding of population genetics, physiology and ecological functions are poorly studied. As stated above, olindiid species have a poor record of elemental composition and energy values. First data on biological parameters of the invasive species *C. sowerbii* (native purportedly to the Yangtze River catchment area) originate from studies in the 1940s to 1960s (Dunham, 1942; Ookawa, 1952; Okuda, 1957, 1960). The most comprehensive examination of biomass and elemental (C and nitrogen; N) composition was carried out by Jankowski (2000). By today, no food web model has explicitly included olindiid medusae despite several known feeding interactions (e.g. Dendy, 1978; Dodson and Cooper, 1983; Jankowski *et al.*, 2005). Environmental conditions were not yet linked to the morphometric and biochemical measurements beyond simple tolerance ranges.

The aim of the present study was to (i) describe the small-scale genetic diversity based on the mitochondrial Cox1 marker of *C. sowerbii* within and among several lakes in southwestern British Columbia in which medusae were previously reported and to contrast these to a population from the other side of the continent (VA) in which hydrozoans have been reported for nearly a century. It was expected that genetic variability within and among the newly introduced British Columbia sites was low compared to the long-established population in VA. Further, we aimed to (ii) report biomass parameters [wet weight (WW), dry weight (DW) and ash-free DW] and elemental composition (C, N and P) data for *in situ*-collected *C. sowerbii* medusae and to compare these among sites as well as own findings with sparsely available literature values for this and other olindiid species. We (iii) explore whether there are stoichiometric changes associated with ontogeny and (iv) estimate the energy content of medusae using published conversion factors to be utilized in future modeling studies.

METHODS

Study sites, specimen collection and environmental parameters

For studying the genetic diversity in isolated *Craspedacusta sowerbii* populations and to be able to draw general conclusions about species stoichiometry, we sampled eight lakes in British Columbia (Canada) between 2 August and 5 September 2021 (five sites on Vancouver Island, one site on Salt Spring Island and two sites at the Sunshine Coast).

In all lakes, *C. sowerbii* was reported in previous years. A more balanced design with the same number of lakes in three regions was not possible, as no medusae were found in other sites. The sampling design, i.e. the frequency of specimen collection and number of specimens collected, was strongly impacted by the site accessibility, medusa size, strength of currents and weather conditions. These factors affected sampling with some selected lakes being sampled more often and thus having more specimens collected than in the others (Table I).

Spanning 150 km between the two most distant lakes, populations were expected to show different genetic signatures if isolation is important. Populations in British Columbia, however, are at the northern distribution range boundary in North America and their introduction happened relatively recently (Marchessaux *et al.*, 2021; Luskow *et al.*, 2021b). Hence, their genetic variability may be expected to be low. If the molecular structure of populations would be similar, one could speak of a metapopulation. Biomass parameters and stoichiometric data derived for the species could then be pooled together, overruling suggested high phenotypic plasticity (Caputo *et al.*, 2021). Some of these lakes are fairly remote and difficult to access, whereas a lake at William & Mary, VA, USA, previously reported to host a *C. sowerbii* population is directly located on the campus. The lake in VA (Crim Dell Lake) was sampled on 17 September 2021. Populations of *C. sowerbii* have been reported from VA for almost a century (e.g. Hamaker and Milne, 1937), suggesting higher genetic variability compared to British Columbia populations. If the suggested North American metapopulation idea is true, then biomass parameters, elemental composition and genetic population structure should be similar despite a distance of ~4000 km between the British Columbia and VA sites. Lake characteristics are summarized in Table I. Lakes investigated ranged in altitude from 17 to 142 m, in surface area from 0.001 to 0.324 km² and in maximum depth from 5 to 14 m (see Ungstad, 2021 for British Columbia lakes, information on Crim Dell Lake, J. Allen, pers. comm.). Most of the lakes are not regularly monitored, so no information about the primary productivity or species composition is available. Jellyfish were either collected on single or multiple occasions within this period, depending on the site accessibility. Logistic limitations restricted the number of specimens possible to analyze per day, so for several lakes with large jellyfish populations, multi-day sampling occurred. We aimed for the largest possible sample size with specimens evenly spread over the jellyfish size range.

Medusae of *C. sowerbii* were gently collected using dip net, cast net and jars from near the surface (top 1 m). Specimens ($N_{\text{total}} = 1385$) were kept in jars filled with lake water on a stand-up paddle board or at the

Table I: Topographic and hydrographic parameters of lakes sampled in August and September 2021

Parameter	Site								
	Crim Dell Lake	Florence Lake	Glen Lake	Hotel Lake	Killarney Lake	Klein Lake	Maltby Lake	McKenzie Lake	Stowell Lake
Latitude (°N)	37°16'14.5"	48°27'30.8"	48°26'14.7"	49°38'23.4"	48°31'41.9"	49°43'47.5"	48°29'47.9"	48°28'56.4"	48°46'54.2"
Longitude (°W)	76°42'49.7"	123°30'45.1"	123°31'20.8"	124°2'44.3"	123°27'23.9"	123°58'7.5"	123°27'9.0"	123°28'51.9"	123°26'39.0"
Region	VA	Vancouver Island	Vancouver Island	Sunshine Coast	Vancouver Island	Sunshine Coast	Vancouver Island	Vancouver Island	Salt Spring Island
Altitude (m)	17	81	67	57	107	142	55	52	77
Surface area (km ²)	0.001	0.084	0.166	0.252	0.034	0.324	0.085	0.036	0.046
Max. depth (m)	3.5	5.5	14	10.6	5.5	41.5	8	5	7.5
Fish stocking	Not stocked	Not stocked	Stocked regularly	Stocked regularly	Not stocked	Not stocked	Not stocked	Not stocked	Stocked regularly
Sampling period	17 Sep 21	2–10 Aug 2021	1–11 Aug 2021	24 Aug–5 Sep 2021	2–24 Aug 2021	5 Sep 21	9–24 Aug 2021	13–24 Aug 2021	5–23 Aug 2021
Surface temperature (°C)	25.3 (1)	24.1 ± 1.0 (4)	24.4 ± 1.0 (5)	20.6 (1)	23.7 ± 1.2 (12)	20.2 (1)	22.7 – 25.5 (2)	24.1 ± 1.0 (3)	23.6 ± 1.8 (3)
Secchi depth (m)		2.9 (1)	4.9 (1)	5.1 (1)	3.6 ± 0.4 (12)	> 10 (1)	4.4 – 4.6 (2)	3.5 ± 0.2 (3)	3.6 ± 0.2 (3)
Chl a (µg L ⁻¹)		0.5 ± 0.4 (4)	0.4 ± 0.1 (5)	1.2 (1)	0.5 ± 0.2 (12)	0.2 (1)	0.2 – 0.7 (2)	0.3 ± 0.0 (3)	0.7 ± 0.5 (3)
N _{jellyfish}	133	7	4	44	842	38	152	50	115

N_{jellyfish} = number of specimens collected per site (N_{total} = 1385); mean ± SD are indicated; number of sampling days for surface temperature, Secchi depth and chlorophyll a concentration (Chl a) is indicated in brackets.

shore until examination in the laboratory. During the field campaign, we searched for polyps on submerged tree branches and stones close to shore, but no specimens were found. Within 4 h after collection, medusae were measured to the nearest mm (i.e. umbrella diameter) using a caliper rule, weighed (WW) on a balance (Fisher Scientific Lab Balance model accu-413, 1 mg precision, or Sartorius BP 211 D balance, 10 µg precision) and stored in groups of individuals with similar size (N = 2–39) in plastic bags at –20°C until further analysis. Grouping took place to ensure sufficient biomass for organic and elemental content analyses. For analyzing the genetic differences between specimens from various lakes, a few specimens from each lake were preserved individually in 95% ethanol. Six specimens (one from each British Columbia site, except for Hotel and Klein Lakes due to logistic problems) were deposited in the Invertebrate Zoology Department Collections, Royal British Columbia Museum in Victoria (catalog numbers: RBCM 021-00057-001–RBCM 021-00062-001).

Along with each medusa sampling, the temperature was measured from the surface to 0.5 m above the lake floor with a YSI Pro30 (Yellow Springs Instruments, OH, USA) or Digi-Sense Thermometer (Cole-Parmer, IL, USA), which was deployed at the center of the lake from a stand-up paddle board. Water transparency was assessed by deploying a 20-cm diameter standard Secchi disk. Water samples (200–250 mL) were taken at 0.5-m depth using optically opaque plastic bottles for chlorophyll a concentration measurement, filtered on pre-combusted glass microfiber filters (Grade F, Sterlitech) and wrapped in aluminum foil frozen until further analysis.

Chlorophyll a concentration measurement

Filters containing pigments from phytoplankton in water samples were placed in 8 mL of 90% acetone at –20°C. After 24 h, the samples were whirl-mixed for 1 min and the filters were withdrawn. The extract was centrifuged for 10 min. Fluorescence was measured with a TD-700 Laboratory Fluorometer (Turner Designs) before and after acidification (Holm-Hansen and Riemann, 1978). The chlorophyll a concentration of the water sample was calculated by relating the chlorophyll a content of the extract to the filtered water volume and was expressed in µg L⁻¹.

Sex determination

The sex of individuals showing developed gametogenic tissues was determined by semi-permanent preparations for optic microscopy (OM) observations. A fragment of gametogenic tissue from each ethanol-preserved medusa was dissected and was placed into a drop of lactic acid on an OM slide before a coverslip was added. The structure of the tissue was observed under a Leica DMLB microscope with Nomarski interference contrast optics, with an OPTIKA C-P20CC digital camera and the OPTIKA PROVIEW image software.

Phylogenetic characterization

DNA extraction and PCR procedures

The total genomic DNA was extracted from ethanol-preserved specimens using the E.Z.N.A. DNA kit (Omega

Biotech, Norcross, USA) following the manufacturer's instructions. The mitochondrial marker *Cox1* was sequenced for characterizing *Craspedacusta* medusae from the examined lakes and for further comparative purposes according to Oualid *et al.* (2019). The *Cox1* marker resolved a higher number of lineages, which was also in accordance with the available sequences in GenBank. The *Cox1* region was amplified using the primers dgLCO1490 5'-GGT CAA CAA ATC ATA AAG AYA TYG G-3' and dgHCO2198 5'-TAA ACT TCA GGG TGA CCA AAR AAY CA-3' (Meyer *et al.*, 2005). The polymerase chain reaction (PCR) used 1 U of MyTaq Red DNA Polymerase (Bioline, Germany), 10 μ M of each primer, \sim 30 ng of genomic DNA and was brought to a final volume of 25 μ L with H₂O. The PCR was carried out using the following cycle profile: initial denaturation at 95°C for 1 min, 40 cycles of denaturation at 95°C for 15 s, annealing at 42°C for 15 s, extension at 72°C for 10 s and a final extension at 72°C for 5 min. The PCR products were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific, Carlsbad, USA) following the manufacturer's instructions before strong amplifications were sent to MACROGEN (Madrid, Spain) for sequencing in both directions.

Phylogenetic analyses

All chromatograms were visualized, and sequence pairs were matched and edited using Sequencher version 4.0. The set of new sequences was assembled with homologous ones from the GenBank of other *Craspedacusta* species. The closest olindiid genus (*Maeotias*) was used as an out-group in the implemented phylogenetic analyses (see relative phylogenetic placement of the genus *Craspedacusta* among olindiids and other selected out-groups in Oualid *et al.*, 2019). The alignments of the set of sequences were carried out using MUSCLE, as implemented in MEGA6 (Tamura *et al.*, 2013). After alignment, the best nucleotide substitution model for each dataset was selected using Modeltest version 2.1.10 (Guindon and Gascuel, 2003; Darriba *et al.*, 2012) according to Akaike's information criterion and hierarchical Likelihood Ratio Test. The *Cox1* dataset extended the tree presented by Luskow *et al.* (2021b) by 42 newly sequenced specimens ($N_{\text{global}} = 110$ *Craspedacusta* sequences and two out-group sequences) with 671 positions, a total of 218 variable and 206 parsimony-informative sites. The small-scale occurrence of distinct genetic lineages of *Craspedacusta* was recently shown in Italy (Morpurgo *et al.*, 2021). Thus, re-sampling of Killarney Lake (initial sampling in 2020; Luskow *et al.*, 2021b) and adding new sites within British Columbia attempted to investigate the haplotype diversity. In the

absence of other *Cox1* sequences from North America, comparable information from VA was highly important. The phylogenetic reconstructions were obtained applying maximum likelihood (ML) and Bayesian inference (BI) methods. ML method was carried out in MEGA6 and was based on K2+I using the nearest neighbor interchange heuristic method and 1000 bootstraps (Bts) replications (Felsenstein, 1985). The BI was carried out in MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) using the model GTR+G (Iset nst = 6 rates = gamma), 10⁷ generations and discarding 25% initial trees.

Weight and elemental composition determination

Frozen samples were dried in an Edwards Modulyo Freeze Dryer at -45°C for 24–72 h or in an oven at 50°C for 60 h. DW of each sample was determined using an analytical balance (Mettler Toledo XP205, 10 μg precision, or Sartorius BP 211 D balance, 10 μg precision). Samples were divided into two groups of similar size-frequency distribution: the first group was used for organic content determination, whereas the second group was used for elemental composition analysis.

Unhomogenized samples of the first group were combusted in a muffle furnace (SYBRON Thermolyne) at 500°C for 24 h, and ash-free dry weight (AFDW) was determined as the difference between DW and ash weight (AW). Blanks were run on empty sampling dishes throughout the analysis. The DW measurement contained residual water (i.e. water bound in collagen and not evaporated during freeze-drying), which leads to an overestimation of the AFDW. The issue of residual water may be more pronounced in collagen-rich cnidarians compared to other phyla. To account for this source of error, a correction factor from marine jellyfish was applied by assuming that 11.7% of the DW was residual water (Larson, 1986). This conversion factor may be different for freshwater species, but currently, there is no alternative evidence.

For the elemental analyses, samples were homogenized with Milli-Q water-cleaned plastic pestle and mortar and afterward returned to 1.5-mL Eppendorf tubes by means of scraping with an Acetone-cleaned stainless-steel spatula. Homogenized samples for carbon (C) and nitrogen (N) analyses were collected in tin caps (8 \times 5 mm) and weighed using an ultra-microbalance (Sartorius Cubis II 2.7S; 0.1 μg precision). Empty tin caps were used as blanks (three blanks per 45 samples), while acetanilide was used as a standard (three standards per 45 samples). Total C and N contents were determined in a CN vario MICRO cube (Elementar, Germany) and expressed per

unit DW. No technical replicates were run because of sample size limitations. The minimum material used in C and N analysis was 0.2 mg. Between 0.9 and 1.8 mg of the homogenized samples (stored in 2 mL Eppendorf tubes) were analyzed for their phosphorus (P) content as orthophosphate after acidic oxidative hydrolysis with 5% H₂SO₄ at the Biologische Anstalt Helgoland, Germany (Grasshoff *et al.*, 1999). Calibration curves for the appropriate ranges were established and always had a $R^2 = 0.99$. Elemental ratios (i.e. C/N, C/P and N/P) were calculated in molar units for each sample.

Energy content estimation

Published conversion factors for diverse sets of marine zooplankton species were used to estimate the energy content of *C. sowerbii* medusae. These were either based on carbon content (C % DW; Platt *et al.*, 1969) or AFDW content (AFDW % DW; Thayer *et al.*, 1973, Båmstedt, 1981). DW-specific energy contents (kJ g DW⁻¹) were compared among conversion methods.

Statistical analyses

Generalized linear models (GLMs) were used to explore the significant (α level of 0.05) effects of size (i.e. umbrella diameter) on WW, DW, AFDW, molar C/N, C/P and N/P values (Gamma error structure, log-link) as well as DW % WW, AFDW % DW, C % DW, N % DW and P % DW (binomial error structure, logit-link) in the R package “lme4” (Bates *et al.*, 2015). For investigating elemental compositions of specimens among sites and regions (Vancouver Island, Salt Spring Island, Sunshine Coast) within British Columbia, non-metric multidimensional scaling (NMDS) analysis was performed, which was followed by an analysis of similarity (ANOSIM) on the dataset with 999 permutations using the “metaMDS” and “anosim” functions from the R package “vegan” (Oksanen *et al.*, 2019) to identify significant differences. One-way analysis of variance (ANOVA) was performed to investigate the difference between AFDW % DW- and C % DW-derived energy contents of jellyfish. Energy contents showed heteroscedasticity and the residuals were non-normally distributed (Shapiro–Wilk normality test and Levene’s test for homogeneity of variance). To meet the assumptions of the ANOVA (i.e. normality of data, homoscedasticity of variances and independence of data), data were log₁₀-transformed. ANOVA was followed by Tukey–HSD *post hoc* tests to pairwise compare elemental contents of specimens collected in various regions and energy contents of medusae derived from three different conversion methods. Analysis of covariance (ANCOVA) was used for investigating slopes of associations of size and site

with log₁₀-transformed WW and AFDW % DW data. All statistical analyses were performed in R (R Core Team, 2022) version 3.6.0.

RESULTS

Environmental conditions

In August and September 2021, *Craspedacusta sowerbii* medusae were collected from eight lakes in British Columbia, Canada, and from one lake in VA, USA (Fig. 1). Medusae occurred in high numbers at all sites but increasingly less often toward mid-September. During this period, surface temperatures were generally high varying between 20 and >25°C (Table I) but decreased over the sampling period (Supplementary Figs S1 and S2). The epilimnion of shallow lakes usually spanned the uppermost 2–3 m separated by a sharp thermocline from the hypolimnion with minimum temperatures of 7.6°C near the lake floor (Maltby Lake). Water transparency at a site generally did not vary throughout the sampling period (Supplementary Fig. S2 and Table I) but showed large differences among lakes (Secchi depth = 2.9 m in Florence Lake; >10 m in Klein Lake). Sub-surface chlorophyll a concentrations were low, relatively constant within a lake but varied among sites and rarely exceeded 1 $\mu\text{g L}^{-1}$ (Supplementary Fig. S2 and Table I).

Sex determination

The sex of the most collected Canadian and US specimens was characterized by the observation of gametes using light microscopy, except for cases of scarce gametogenic structure development. In two Canadian lakes (Glen and McKenzie Lakes), sexes could not be determined, as well as in some of the specimens from Florence and Crim Dell Lakes. In all remaining cases, Canadian examined specimens were male, whereas US-examined specimens were female (Supplementary Fig. S3). Available samples for this study were biased toward British Columbia sites (male populations), with male specimens originating from eight lakes and female specimens originating from one lake. Given most often unisexual medusa populations in non-indigenous habitats worldwide and previous evidence from Killarney Lake, we suggest also unisexual populations in the studied lakes. In the following, all specimens were analyzed together, leaving an inter-sex comparison for future efforts.

Phylogenetic analyses

Forty-two of the 49 ethanol-preserved specimens from which DNA was extracted were successfully amplified



Fig. 1. Location of sampling sites in (A) British Columbia (Canada) and (B) VA (USA). Sites in British Columbia are divided by region (Sunshine Coast, Salt Spring Island, Vancouver Island).

in the PCRs carried out to obtain the *Cox1* marker (8 from Crim Dell Lake, 4 from Florence Lake, 3 from Glen Lake, 2 from Hotel Lake, 2 from Killarney Lake, 6 from Klein Lake, 6 from Maltby Lake, 8 from McKenzie Lake and 3 from Stowell Lake). Thus, between two and eight sequences from each of the sampled lakes were available for this comparison.

The *Cox1* trees obtained by BI and ML showed the same topology (Fig. 2), and the only difference was related to the unstable relationship of previously published

Craspedacusta sequences from Switzerland and Japan (Osaka, see below).

The BI and ML trees placed together all Canadian and US sequences in a strongly supported polytomy [Bts 100, posterior probability (PP) 0.99], together with South American (Chile), Japanese (Nagano), European (Sicily, Greece), Indian (Kerala), Singaporean, Czech and Eastern Chinese (Hubei province) sequences. A sequence from an unknown origin (GenBank Accession LN901194) was also aligned with American (Canada– USA–Chile)

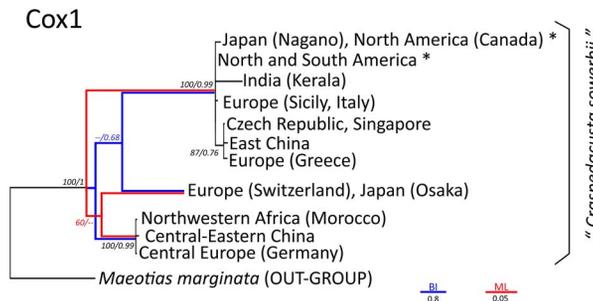


Fig. 2. Relationships of known Cox1 sequences of *Craspedacusta sowerbii* grouped by geographic area using the outgroup *Maeotias marginata* as outgroup. Differences between BI (blue branches) and ML (red branches) methods are indicated. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Supported values of the nodes (Bts/PP) are shown. (–) clade not supported by BI or ML method. Asterisks (*) indicate where the new sequences specimens are grouped.

sequences. Both inference methods showed a well-supported grouping (Bts 100, PP 0.99), including Central Europe (Germany), Northwest African (Morocco) and Central-Eastern Chinese sequences. A distinctly divergent sequence present in Europe (Switzerland) and Japan (Osaka) was the sister group of the first mentioned set of sequences in the BI tree (with low-moderate support, PP 0.68, blue lines), or the sister group of the second mentioned set of sequences in the BI tree (with low support also, Bts 60, red lines).

The material sequenced in this study [Canada and USA (VA)], belonged to a geographically well-distributed grouping of sequences (America, Europe and Asia). One of the two Canadian sequences is also present in Japan (Nagano), while the other Canadian sequence is also found in the US (VA) and Chile. The grouping in which the New World material is included is 15.9% distant from the Switzerland-Japan (Osaka) sequence and 15.5% distant from the grouping including Germany-Morocco + Central-Eastern China set of sequences. This last one is 12.4% distant from the Switzerland-Japan (Osaka) sequence. The German sequences are only one base different from the Moroccan sequences, and both are 0.3% distant from the Central-Eastern Chinese sequences. The difference between both Canadian sequences involves 3 bases (0.5%).

Biomass parameters (WW, DW and AFDW)

Individual biomass of 1385 specimens ($N_{\text{female}} = 123$, $N_{\text{male}} = 1252$) ranging from 2 to 25 mm umbrella diameter was measured. All biomass parameters, i.e. WW, DW and AFDW, increased significantly ($P < 0.001$) with size (Fig. 3A, B and D; Table II). Analyzing the effect of site on WW and AFDW % DW of medusae, correlations

were hampered by a little size overlap between specimens collected in various lakes (Supplementary Fig. S4). However, ANCOVA indicated an insignificant effect between Maltby and Stowell Lakes on WW slopes ($P = 0.299$) and AFDW % DW slopes ($P = 0.186$). Thus, we pooled data from all sites and derived global equations for *Craspedacusta* medusae (Table II). The water content of *C. sowerbii* medusae varied between 98.5 and 99.7% and was not affected by size ($P = 0.985$; Fig. 3C and Table II). The organic content of medusae (corrected AFDW % DW = 69.0–95.7%) varied considerably with size, but its change was not statistically significant ($P = 0.770$; Fig. 3E and Table II).

Elemental composition

Carbon (C), nitrogen (N) and phosphorus (P) contents were only measured for Canadian (male) specimens due to technical restrictions at the US site. Female specimens ($N = 123$ in 12 samples grouped by size) from the site in VA were used together with samples from British Columbia for organic content analysis. Elemental contents (expressed as percent of DW) of medusae are depicted in Fig. 3F–H. C % DW ranged between 41.9 and 53.6%, N % DW varied between 7.6 and 11.0%, while P % DW spanned 0.6–1.4% (Table II). Elemental contents of medusae did not vary significantly among three regions (Sunshine Coast, Salt Spring Island, Vancouver Island) within British Columbia ($P \geq 0.672$), except for C % DW at the Vancouver Island sites ($P \leq 0.009$) and N % DW at the Salt Spring Island site ($P \leq 0.002$, Fig. 4). A NMDS ordination and ANOSIM showed that the stoichiometry of medusae collected in three regions within British Columbia was not significantly impacted either by region ($R = 0.024$, $P = 0.297$) or by site ($R = 0.040$, $P = 0.144$, Fig. 4). C % DW, N % DW and P % DW varied with size, but trends were not significant ($P = 0.941$ – 0.963 , $N = 177$ – 179 , Table II). Predicting one elemental content from another (e.g. estimating nitrogen content from carbon content) is not recommended in *C. sowerbii* because of high variability and no obvious trends (Fig. 5). Molar C/N values decreased with size ($P < 0.001$; Table II), whereas molar C/P and N/P values showed no significant change with medusa umbrella diameter ($P = 0.249$ and 0.852 , respectively; Fig. 3I–K).

Energy content

The estimated energy contents using three equations (two of them using AFDW % DW data and one using C % DW data) aligned well (Supplementary Fig. S5). However, significant differences between methods were apparent ($P < 0.001$). The conversion method from Platt *et al.*

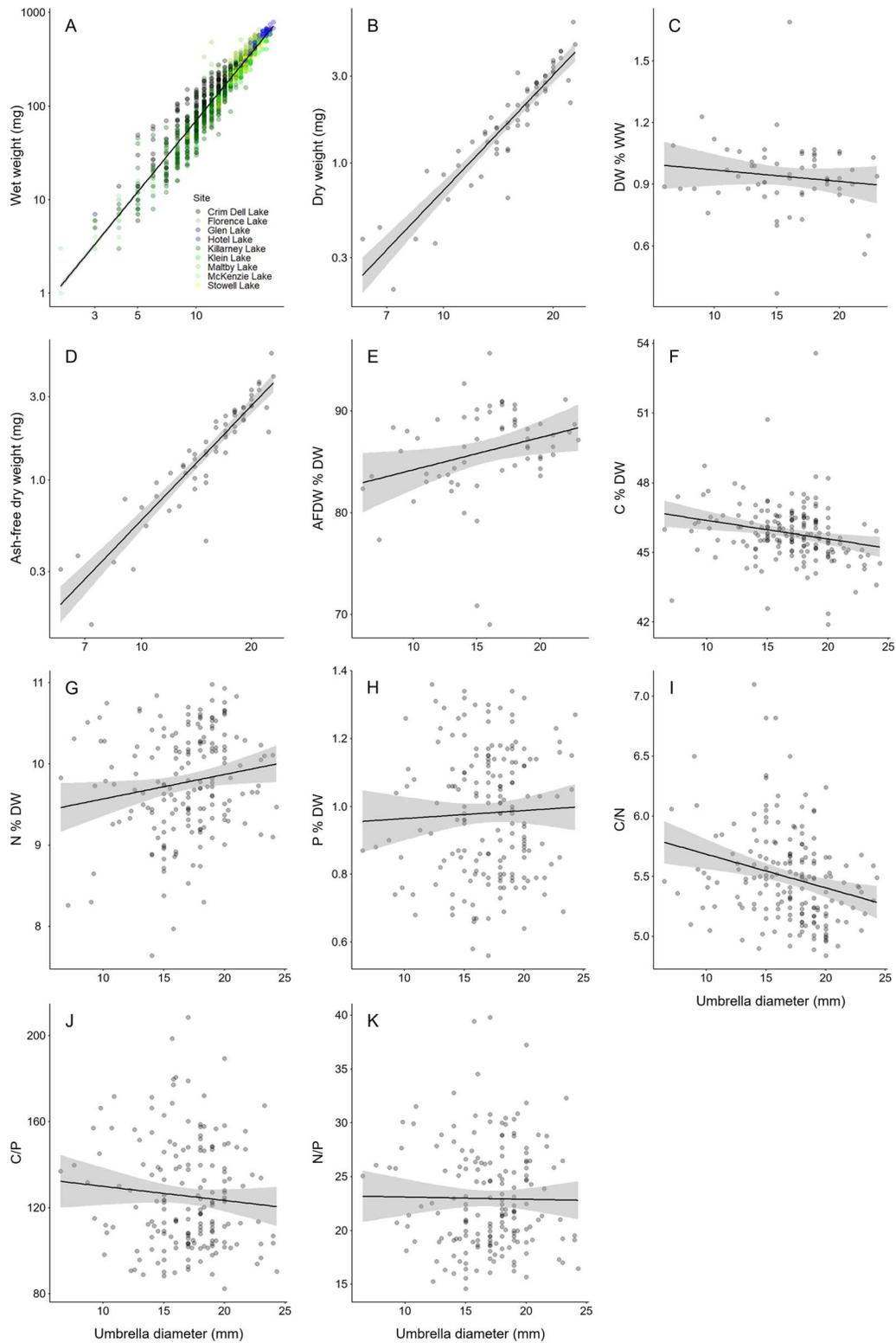


Fig. 3. *Craspedacusta sowerbii*. Relationship of (A) WW, (B) DW, (C) DW % WW, (D) AFDW, (E) organic content (AFDW % DW), (F) carbon content (C % DW), (G) nitrogen content (N % DW), (H) phosphorus content (P % DW), (I) molar C/N value, (J) molar C/P value and (K) molar N/P value with size (umbrella diameter). Sites are indicated by color. Note: Some relationships are shown on a log–log scale. Linear regression lines are shown with 95% confidence intervals (gray shading). Regression equations are given in Table II.

Table II: Regression equations of associations shown in Fig. 3

Sex	Explanatory variable	Range (mm)	Response variable	Range	Regression equation	R^2	$N_{\text{jellyfish}}$	t -value	z -value	P -value
F + M	<i>d</i>	2.0–25.0	WW	1–795 mg	$WW = 0.206d^{2.535}$	0.925	1 375	93.5		< 0.001
F + M	<i>d</i>	6.0–23.0	DW	0.2–6.0 mg	$DW = 0.005d^{2.114}$	0.816	61	19.49		< 0.001
F + M	<i>d</i>	6.0–23.0	DW % WW	0.3–1.5%	$DW \% = -0.005d + 0.906$	0.017	61		-0.02	0.985
F + M	<i>d</i>	6.0–23.0	AFDW	0.2–5.3 mg	$AFDW = 0.005d^{2.158}$	0.829	61	20.79		< 0.001
F + M	<i>d</i>	6.0–23.0	AFDW % DW	69.0–95.7%	$AFDW \% = 0.317d + 81.061$	0.083	61		0.29	0.77
M	<i>d</i>	6.5–24.3	C % DW	41.9–53.6%	$C \% DW = -0.080d + 47.185$	0.049	179		-0.07	0.941
M	<i>d</i>	6.5–24.3	N % DW	7.6–11.0%	$N \% DW = 0.030d + 9.267$	0.026	179		0.05	0.963
M	<i>d</i>	6.5–24.3	P % DW	0.6–1.4%	$P \% DW = 0.002d + 0.952$	0.001	177		0.05	0.961
M	<i>d</i>	6.5–24.3	C/N	4.8–7.1	$C/N = -0.028d + 5.968$	0.062	179	3.41		< 0.001
M	<i>d</i>	6.5–24.3	C/P	82.3–208.7	$C/P = -0.657d + 136.570$	0.008	177	1.16		0.249
M	<i>d</i>	6.5–24.3	N/P	14.6–39.8	$N/P = -0.021d + 23.327$	0.001	177	0.19		0.852

Ranges of explanatory and response variables; R^2 , correlation coefficient; $N_{\text{jellyfish}}$, number of specimens collected per site; P -, z - and t -values from GLMs are shown; F, female; M, male; *d*, umbrella diameter; C, carbon; N, nitrogen; P, phosphorus. Elemental ratios are given in molar units. Corrected AFDW % DW assumed residual water is 11.7% of DW (Larson, 1986). Significant slopes ($P < 0.05$) are indicated in bold.

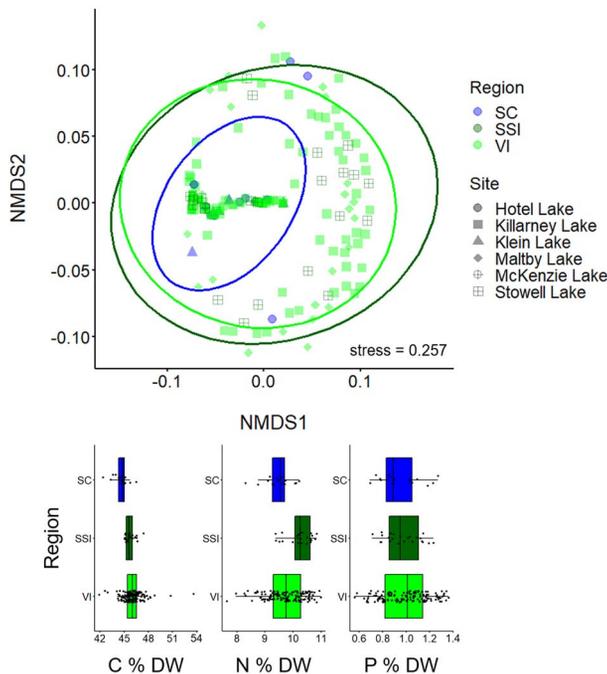


Fig. 4. *Craspedacusta sowerbii*. NMDS ordination of medusae sampled in eight sites (three regions) in British Columbia in August and September 2021 according to their elemental (C, N and P) composition (upper panel). Similarity between medusa stoichiometries was calculated by means of Bray-Curtis measurements based on family densities. Side-by-side box plots of C, N and P contents of medusae split by region (lower panel). SC, Sunshine Coast (Hotel and Klein Lakes); SSI, Salt Spring Island (Stowell Lake); VI, Vancouver Island (Killarney, Maltby and McKenzie Lakes); line, median; box, inter-quartile range (IQR); whiskers, max/min value $\leq 1.5 \times$ IQR above/below box.

(1969) used C % DW in contrast to the other two methods and yielded the highest weight-specific energy contents ($28.2 \pm 0.8 \text{ kJ g DW}^{-1}$, mean \pm SD). Energy contents

derived from the conversion equation given by Båmstedt (1981) are positioned between contents based on the other conversions. All conversion factors are based on marine mixed macrozooplankton, but the one by Båmstedt (1981) included some gelatinous zooplankton (GZ) species. In the following, only the energy conversion equation based on AFDW % DW given by Båmstedt (1981) was used. Weight-specific energy contents of *C. sowerbii* ranged from 19.0 to 27.0 kJ g DW^{-1} . As shown in Supplementary Fig S6, the weight-specific energy content of medusae increased significantly ($P = 0.042$), but with a low slope ($a = 0.022$), whereas the total energy content (i.e. product of weight-specific energy content and individual DW) had a high ($a = 1.022$) and significant slope over the given DW range ($P < 0.001$). Energy contents along with AFDW % DW and molar C/N and C/P values of *C. sowerbii* obtained in this study were compared with literature values for marine hydrozoans (Fig. 6 and Supplementary Table SI), other members of the family Olindiidae (Tables III) as well as with other freshwater zooplankton.

DISCUSSION

Phylogenetic relationships of Canadian and American specimens

In agreement with previous studies (Oualid *et al.*, 2019; Luskow *et al.*, 2021b; Peterson *et al.*, 2022), Cox1 analyses showed globally at least three main *C. sowerbii* lineages; the newly sequenced North American specimens in our study clustered with the South American, Japanese, Southern European (Sicily, Greece), Indian, Czech and East Chinese (Hubei) counterparts. The relationships of the Switzerland-Japanese (Osaka) sequence remain pending a more comprehensive molecular sampling of this and

Table III: Biomass parameters, elemental composition and energy content of various species of the family Olindiidae

Species	Sex	DW % WW	AFDW % DW	C % DW	N % DW	P % DW	C/N	C/P	N/P	Energy content (kJ g DW ⁻¹)	Reference	
<i>Craspedacosta sowerbii</i>	F	0.8 ± 0.1 (10)									Dunham (1942)	
	NS	1.8 ± 0.8 (37)									Ookawa (1952)	
	F	1.1 ± 0.6 (150)	90 (NS)								Okuda (1957)	
	NS	1 (NS)	90.2 ⁺ (NS)								Okuda (1960)	
	F	0.4-1.2 (NS)									Fleming and Hazelwood (1971)	
	F	0.7 (210)	90.5 (NS)								Ludwig (1977)	
	NS	1.0 ± 0.5 (89)		29.8 ± 1.1 (24)	8.0 ± 1.1 (24)	2.0 ± 0.2 (12)	4.4 (24)	39 (12)	9 (12)		Jankowski (2000)	
	F+M	0.8 ± 0.2 (61)	86.1 ± 4.5 (61)	45.8 ± 1.3 (179)	9.8 ± 0.6 (179)	1.0 ± 0.2 (177)	5.5 ± 0.4 (179)	125.4 ± 25.2 (177)	23.0 ± 4.9 (177)		24.1 ± 1.4 (61)	This study
	NS	4.0 ± 0.1 (11)	31.0 ± 0.7 (8)	8.6 ± 0.8 (6)	2.5 ± 0.1 (6)		4 (6)				Larson (1986)	
	NS			7.5 (1)	1.9 (1)		4.6 (1)				Luskow et al. (2021a)	
<i>Gonionemus vertens</i>	NS	4.6 ± 0.1 (18)	48.0 ± 3.2 (8)	13.7 ± 0.1 (3)	4.1 ± 0.1 (3)		3.9 (3)				Larson (1986)	
	NS						5.3 (10)	342.9 (10)	64.2 (10)		Malzahn et al. (2010)*	
<i>Limnocooida tanganyicae</i>	F+M	0.8 (10)									Bouillon (1957)	
	NS		78.6 ± 8.9 ⁺ (33)								Salonen et al. (2012)	
<i>Olindias sambaquiensis</i>	NS	6.4 ⁺ (~80 ⁺)	46.4 ⁺ (~80 ⁺)								Chiaverano et al. (2004)	
	NS	3.2 ± 0.4 (4 ⁺)	37.0 ± 0.0 (4 ⁺)								De Barba et al. (2016)	

F, female; M, male; NS, not specified; C, carbon; N, nitrogen; P, phosphorus. Corrected AFDW % DW assumed residual water is 11.7% of DW (Larson, 1986). Molar elemental ratios are shown; *, medusae were raised in the laboratory; +, data read from original figures or obtained upon request from the author. Number of samples is indicated below values in brackets. Mean ± SD are indicated.

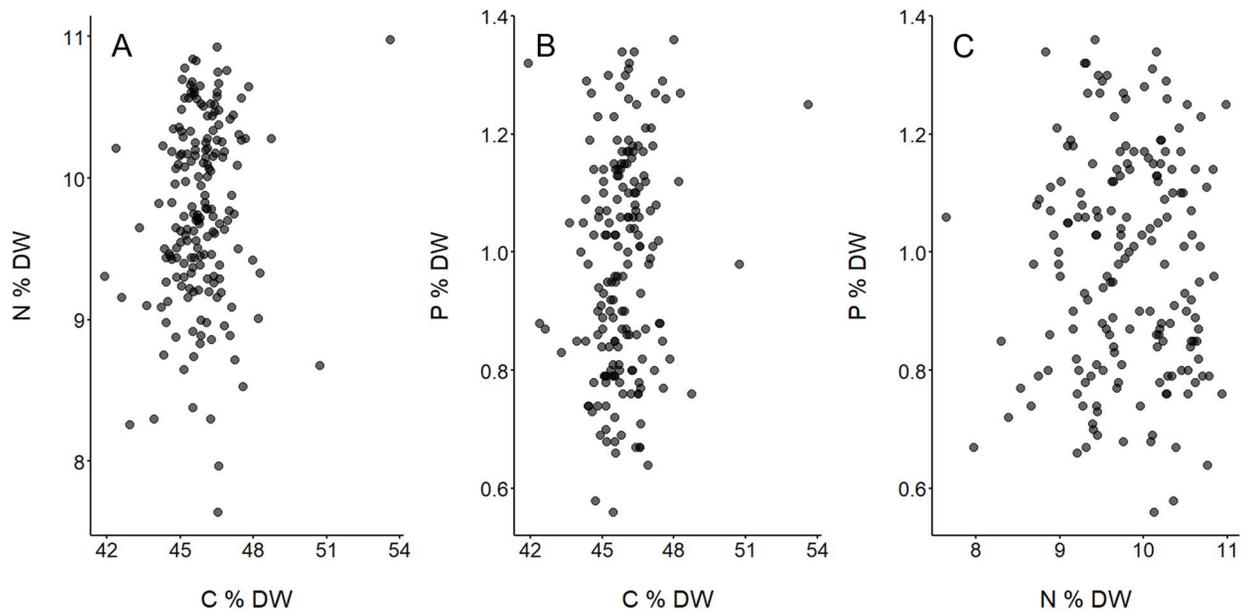


Fig. 5. *Craspedacusta sowerbii*. Relationship between (A) C and N, (B) C and P and (C) N and P expressed as percent of DW ($N_{\text{jellyfish}} = 179$ and 177, respectively).

other localities worldwide. The unstable placement of Switzerland-Japanese (Osaka) *Cox1* sequences has been already pointed out (Oualid *et al.*, 2019; Lüsrow *et al.*, 2021b; Peterson *et al.*, 2022), suggesting three different lineages in Europe, two in Central Europe (Germany and Switzerland) and a third in Southern Europe (Sicily and Greece). Regarding the 16S available information, the analyses of this marker consider mainly two lineages: (i) Germany + Switzerland + North Italy related to Morocco, and (ii) South Italy (Sicily) related to South America + USA + East China (Hubei) (Morpurgo *et al.*, 2021). Available ITS information shows a more complex view when sequences attributed to *C. ziguiensis*, *C. sinensis*, *C. brevinema* and *C. kiatingi* (all from China) are included in the analyses (Oualid *et al.*, 2019; Lüsrow *et al.*, 2021b). According to Lüsrow *et al.* (2021b), both *C. sowerbii* main lineages are present in China plus some countries of the Old and New Worlds. Despite the presence of different lineages of *C. sowerbii*, the nomenclatural solution about which of these lineages must be considered *C. sowerbii* is pending further molecular sampling in the type location in South England (see Oualid *et al.*, 2019).

To not repeat previously discussed ideas on the known global phylogenetic relationships of all available *C. sowerbii*-sequenced materials, we will focus mainly on the North American localities. Even within the same main clade, regarding the *Cox1* marker, only two slightly different sequences have been found in the Americas; one of them is present in Canada, the USA and Chile (Fuentes *et al.*, 2019; Lüsrow *et al.*, 2021b; present study), whereas a

second one has presently only been found in Canadian lakes (this study) and recently in Japan (Nagano) (Peterson *et al.*, 2022). Both sequences differ at three bases. In Canadian (British Columbian) lakes, the most widely distributed haplotype (shared with the USA and Chilean sites) is present in Florence and Glen Lakes, while an exclusive Canadian haplotype that was also recently found in Japan (Nagano) is only present in Hotel, Killarney, Klein, Maltby and Stowell Lakes. In all these lakes, a single haplotype was found. However, in McKenzie Lake, both haplotypes were found (seven of the former and one of the latter).

The detection of *C. sowerbii* by medusa sighting has a very narrow environmental condition and temporal window. Additional next-generation sequencing technologies, such as environmental DNA (eDNA), have been proven successful in detecting *C. sowerbii* (e.g. Blackman *et al.*, 2022). Such detections can be produced even when no medusa sightings exist and are potentially beyond the medusa developing period as the polyp stage is present year-round. eDNA detections could be extremely useful in addressing those water bodies where a more detailed monitoring program could be developed. In these surveys, changes in the haplotype, i.e. the addition of new haplotypes or changes in haplotype ratios, or sex structures, i.e. maintenance of a single sex per water body or apparition of both sexes, should be monitored to better evaluate the potential risks if a more diverse (genetic and sexual) population is reached.

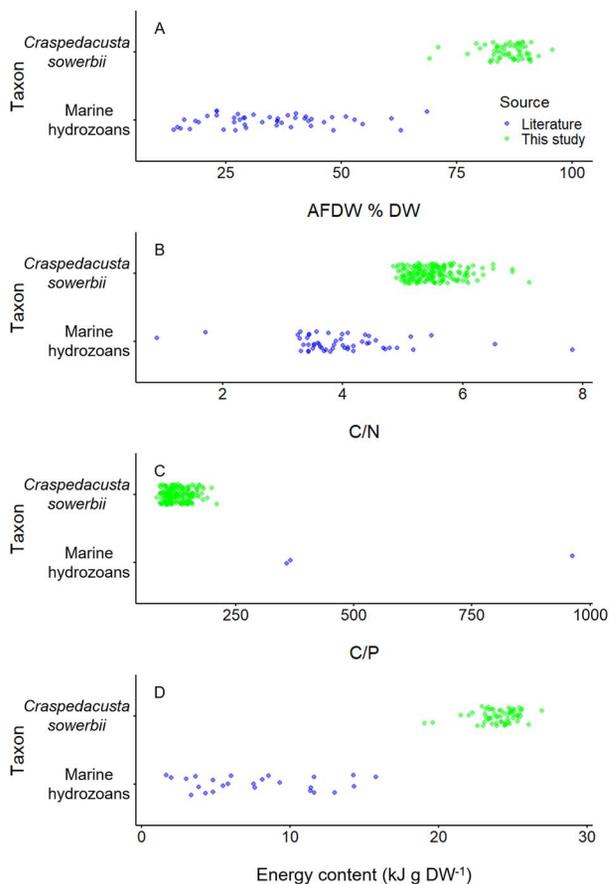


Fig. 6. Comparison of (A) organic content (corrected AFDW as a percentage of DW, AFDW % DW), (B) molar C/N value, (C) molar C/P value and (D) energy content (conversion from Bämstedt, 1981) between *C. sowerbii* in the present study (both sexes considered) and previously published values for marine hydrozoans (Supplementary Table S1).

At present, in all cases where sex could be determined, only male medusae were detected in the Canadian lakes (Corry, 1994; Luskow *et al.*, 2021b), whereas all examined specimens from Crim Dell Lake (USA) were female. For two Canadian sites (Glen and McKenzie Lakes), gametogenic tissues were not developed sufficiently to determine sex. McKenzie Lake may become one of the most interesting targets for further investigations as two haplotypes are present and no sex determination has been achieved in the present study.

Water and organic content of *C. sowerbii* medusae

Unlike most jellyfish, *C. sowerbii* occurs exclusively in freshwater systems, which has implications for its salt content and DW dependent variables. In marine jellyfish, salt can contribute >80% of the DW (Hubot *et al.*, 2022). While the water content in marine and brackish

water olindiid species is typically between 94 and 97%, *C. sowerbii* has an even higher water content reaching around 99%. This agrees with earlier studies (Table III) and values for the freshwater hydrozoan *Limnocnida tangeriicae* (99.2%; Bouillon, 1957). Jellyfish are known to be osmoconformers (Mills, 1984), and thus considerably lower ambient freshwater salt content contributes little to the species' DW. A decrease in DW as a percent of WW with size was previously reported for *C. sowerbii* (Fleming and Hazelwood, 1971). This trend was not supported in our study, as DW % WW varied insignificantly over the size range. The DW % WW even slightly increased, which may be a result of gametogenic tissue development.

The organic part of the DW (i.e. AFDW) is approximately double in *C. sowerbii* and *L. tangeriicae* (79–90%, Table III) compared to *Eperetmus typus* (31%; Larson, 1986), *Gonionemus vertens* (48%, Larson, 1986) and *Olindias sambahiensis* (46 and 37%; Chiaverano *et al.*, 2004; De Barba *et al.*, 2016). This is further supported by values of a wide range of marine hydrozoans (14–87%) and again a consequence of less salt contributing to lower inorganic part of the DW (i.e. AW) in freshwater species. A size-related change in organic content was found to be insignificant in the present study. Similarly, Arai (1986) did not find a change in the organic content of *Aequorea victoria* with increasing size. More recently, Luskow *et al.* (2021a) found size effects of the organic content of nearly all investigated marine species, but only a few of them were significant.

Stoichiometry of medusae and implications for food webs

It can be assumed that *C. sowerbii* is able to maintain some level of homeostasis as has been shown experimentally for some marine gelatinous zooplankton (GZ) species (Chen and Li, 2017; Schoo *et al.*, 2010). Hence, measurements of elements and their ratios from field samples can be attributed to species properties rather than to external conditions. Carbon (C) and nitrogen (N) contents in *C. sowerbii* are 2- to 5-fold higher than in other marine olindiid species, such as *E. typus* (Larson, 1986; Luskow *et al.*, 2021a) and *G. vertens* (Larson, 1986), but compare well, albeit on the higher end, with data shown by Jankowski (2000). The phosphorus (P) content of medusae in our study was only half (~1% of DW) of what was reported by Jankowski (2000). We had a 10 times larger sample size and slightly broader size range than the previous investigation. Unfortunately, there are no P content data published for any other olindiid species. Marine GZ have typically P contents that are 2- to 10-fold lower than what is reported for *C. sowerbii* (Luskow *et al.*, 2022). This difference is likely explained by the

variable contribution of salt to the DW. It is noteworthy that this trend seems well supported, but there are only a few available P content values for GZ and more data for marine and other olindiid species are required to draw conclusions.

The C/N value of *C. sowerbii* medusae (5.5 ± 0.4 , mean \pm SD) is the highest reported for olindiid species and is above values for most marine hydrozoans. This indicates that *C. sowerbii* is relatively rich in lipids compared with other hydrozoans. Further, the C/N value was shown to decrease significantly with size, pointing toward a decrease in the importance of lipids during growth. As reviewed by Lüsrow *et al.* (2021a), most GZ species do not exhibit a change in C/N value over their size ranges. Thus, the trend seen for male *C. sowerbii* medusae awaits an explanation. Other molar ratios (C/P and N/P) did not vary with size and fell in between previously reported values for *C. sowerbii* and *G. vertens* (Jankowski, 2000; Malzahn *et al.*, 2010). On a broader scale, C/P values of *C. sowerbii* rank at the lower end of the range reported for (predominantly marine) GZ (Lüsrow *et al.*, 2022).

Compared to marine hydrozoans, *Craspedacusta* medusae are relatively rich in C, N, and especially P, which makes them, stoichiometrically speaking, more similar to P-rich cladocerans than to N-rich copepods (Sommer and Stibor, 2002). There is a consensus that primary production in lakes (and freshwater systems in general) is more often P-limited, whereas marine systems are N-limited, and that seston C/P values in lakes are generally higher than in marine environments (Sternner and Elser, 2002). The main crustacean prey in marine systems (i.e. copepods) have much lower P content than cladocerans in lakes (Sternner and Elser, 2002), which makes copepods superior under P limitation. The mean molar C/P value of *C. sowerbii* medusae of 125 is located in between copepods (typically 250–350) and cladocerans (typically 75–100; van de Waal *et al.*, 2010), indicating that both prey groups are their stoichiometrically suitable prey. This is supported by the results of several feeding experiments (e.g. Dodson and Cooper, 1983; Jankowski *et al.*, 2005). A recent study showed that high phenotypic plasticity in *C. sowerbii* is apparently associated with the type and quality of their prey (crustaceans vs. ciliates; Caputo *et al.*, 2021), supporting a flexible impact on lower trophic levels. This high phenotypic plasticity may also explain the lack of genetic differences between North American *C. sowerbii* populations. Further, *C. sowerbii* medusae and juvenile fish have highly comparable C/P values (van de Waal *et al.*, 2010), which is indicative of potential food competition. However, as adult fish have a lower and more confined C/P range, medusae may not be the ideal prey to fulfill their nutrient requirements. Future research will need

to address how (qualitatively and quantitatively) these trophic interactions will be affected by current stressors such as eutrophication, warming and biodiversity loss.

Medusa energy content and food web implications

Our calculated energy content data for *C. sowerbii* are the first-ever reported values for freshwater jellyfish or olindiid species. We need to remember that these energy contents were estimated from AFDW % DW and a generic zooplankton conversion equation (Båmstedt, 1981), introducing some bias for which we cannot account. Direct energy content measurements via bomb calorimetry would be desirable to confirm our estimates. However, our organic content data were highly similar to values in earlier studies (Okuda, 1957, 1960; Ludwig, 1977), indirectly supporting the energy content calculations. Doyle *et al.* (2007) calculated the energy contents for three scyphozoan jellyfish species using proximate composition and bomb calorimetry and found very good agreement between both techniques, further lending support to our energy content estimations.

The energy content of *C. sowerbii* is remarkably higher than in all marine hydrozoans (Lüsrow *et al.*, 2021a) but similar to brackish water copepods (Chang and Lei, 1993), rotifers (Román Reyes *et al.*, 2017) as well as freshwater copepods and cladocerans (Vijverberg and Frank, 1976), ranging between 20 and 27 kJ g DW⁻¹. The weight-specific energy content of medusae increased significantly with size, which could be explained by an advanced development of gametogenic tissues. Nevertheless, upon grouping all marine GZ taxa, the weight-specific energy content decreased significantly with size (Lüsrow *et al.*, 2021a). This, however, is not surprising as the species-specific differences are masked when a wide size range (6–610 mm) is considered.

The high weight-specific energy content together with fast growth, comparably large size and low mobility, common for most GZ species (Madin and Harbison, 2001), may potentially pose *C. sowerbii* medusae as an energetically (but not stoichiometrically) attractive prey for higher trophic-level predators, including fish, amphibians and reptiles. However, very few qualitative observations of medusa consumption in lakes currently exist (Dodson and Cooper, 1983; T. Peard pers. comm.). Future research efforts are needed to disentangle *in situ* trophic relationships via biochemical markers, molecular tools and quantitative visual observations. Such knowledge will be vital to informing food web and biogeochemical models exploring the effects of annually recurrent summer/autumn freshwater jellyfish blooms (Pauly *et al.*, 2009; Hays *et al.*, 2018; Lamb *et al.*, 2019). On the contrary, the high

growth rates and fast gametogenic tissue maturation (a matter of weeks) require high feeding rates, assimilation efficiency and/or low respiration rates. All these traits are typical for jellyfish (Rüsgård and Larsen, 2022), however, have not been studied in detail for *C. sowerbii*. Currently, energy channeled into gametogenic tissue maturation in the unisexual medusa population is not translated into genetic recombination and production of larvae securing a genetically diverse polyp population. Hence, medusa production and shunting of energy away from other food web components does not seem to be efficient or sustainable, given mostly unknown predator–prey interactions of the medusae. This, however, may quickly change with the arrival of polyps and/or frustules of the second sex, requesting continuous monitoring of known unisexual populations in North America.

CONCLUSION

In North America, two closely related sequences occur, one shared by the Canadian and Japanese specimens, while another one is widely present across the Americas. The organic and all elemental contents of *C. sowerbii* medusae were higher compared to the marine jellyfish species. Estimated energy content of *C. sowerbii* was 20–27 kJ g DW⁻¹, which is 2-fold higher than for marine hydrozoans and compared well with cladocerans, copepods and rotifers. The estimated C/P values indicated cladocerans and copepods are suitable prey for *C. sowerbii* and that jellyfish may compete with juvenile fish to fulfill similar nutrient demands. Freshwater jellyfish were stoichiometrically and energetically different from marine hydrozoans, indicating higher importance in freshwater food webs than previously thought. As highlighted in recent modeling studies (Marchessaux et al., 2021, 2022), the global distribution range, as well as climatic conditions allowing for the reproduction of *C. sowerbii* across seasons, will expand and increase their impact on food webs and nutrient cycles. At present, we are just beginning to understand the biology and ecology of *Craspedacusta* and other freshwater hydromedusa species.

DATA AVAILABILITY

Biomass and elemental composition data have been deposited in PANGAEA (<https://doi.pangaea.de/10.1594/PANGAEA.947306>). All haplotype sequences have been deposited in the GenBank database under the accession numbers ON606476–ON606485.

SUPPLEMENTARY DATA

Supplementary data is available at *Journal of Plankton Research* online.

ACKNOWLEDGEMENTS

We are grateful for the support of Alexis A. Bahl, Polina Orlov, Genevieve Lawrie, Martha Young, Maria Clagett and Alexis Reece during the sample collection and laboratory work. Thanks to Deborah K. Steinberg, who granted us lab space at VIMS. We appreciate that John and Carmel Thomson as well as Bradley Johnson, gave permission to sample Maltby and McKenzie Lakes, respectively, from their properties, and we acknowledge Capital Regional District (CRD) Regional Parks for permitting sample collection at Killarney Lake. We thank Ursula Ecker, Julia Haafke and Ragna Bergmann for their help in the laboratory with elemental composition measurements. We acknowledge the raw data made available by Luciano Chiaverano, Kalevi Salonen and Charrid Resgalla Jr as well as comments made by Henry Choong, two anonymous reviewers and the editor on an earlier version of the manuscript.

FUNDING

International Doctoral Fellowship (IDF) scholarship granted by the University of British Columbia (to F.L.); Natural Sciences and Engineering Research Council (NSERC) Discovery (Grant RGPIN-2014-05107 to E.A.P.).

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