

Particles Assembling and Fluid Dynamic Features Lend to Daphnia's Feeding Mechanism: A Commentary Review

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Abstract

Since the early 1920's, the feeding mechanism of Daphnia has been defined as a sieving action. The use of cinematographic methodology and fluid dynamics analyses confirmed a historical turning point from sieving to an assembly mechanism. Filtration and capillarity were dismissed, whereas fluid dynamics principles supported the assembly hypothesis.

Keywords

Daphnia, Feeding, Filtration, Assembling, Evolutionary Advantage

1. Introduction

Natural and industrial extraction and sorting of suspended particles from a fluid microflow are today's challenges, not only for researchers in microfluidics but also for limnologists interested in the feeding mechanisms of the freshwater zooplankter Daphnia. Daphnia spp., a microcrustacean of 1 to 3 mm (about 0.12 in) size, features four pairs of appendages (2P3 and 2P4) that are covered by the two parts of a folded carapace. These appendages move rhythmically, beating at a frequency around 10 Hz and generate an in/out-microcurrent flow system to extract suspended particles (algae, protozoa, bacteria, detritus, etc.) for food.

Daphnia is a freshwater crustacean that is globally widely distributed, belonging taxonomically to the order of Cladocera (water fleas). The thoracic and abdominal soft tissues are covered by a bivalved appearance, which is actually a single folded piece ventrally gaped. The soft tissues of digestion, reproduction, thoracic appendages and others are located between the two carapace valves. Among those, two trunk limbs, defined as P3 and P4, are responsible for the internal water cir-

culation. The functional role of P3 and P4 is respiration (gas exchange) and feeding. The internal water circulation is operated by rhythmic up-downward beats of P3 and P4 trunk limb appendages. An inappropriate determined role definition of the trunk limbs, P3 and P4 (**Figure 1**), as sieve organs was totally accepted for more than a century. Nevertheless, recent cinematographic methodological research denied filtering activity [1]. The long research history granted them a definition of filters which is in contradiction with the widely accepted “Solid walls” terminology [2]-[4]. The definition of their role in *Daphnia*'s feeding mechanism as filters was based on a structural anatomy paradigm initiated in the early 1920's [5] and strictly concluded after a thorough morpho-anatomical study during the 1990s [6]-[8]. Recent analyses of slow motion Cinematographic filmed *Daphnia*'s feeding action and solid photos at a time interval of 10 milliseconds (MS) denied filtration activity, whilst particle assembling is a suitable definition of the food particles collection. The technical methodological background was documented earlier [9]-[11] whilst aspects of Fluid Dynamics are considered in the present paper.

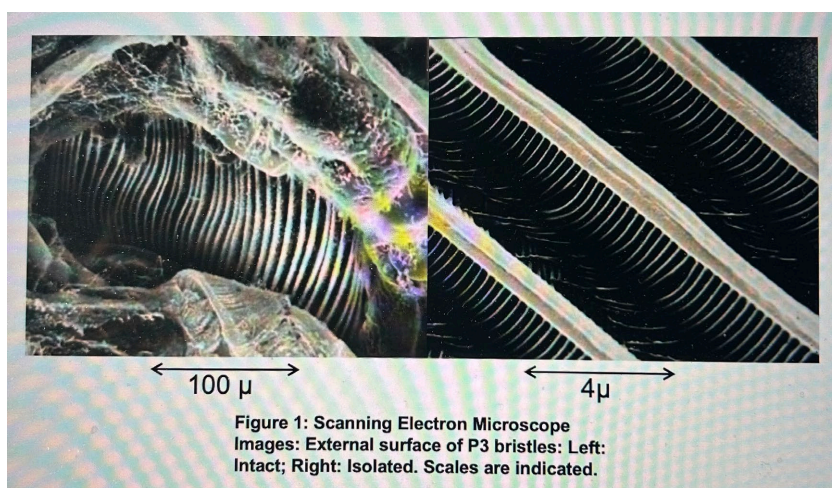


Figure 1. Scanning electron microscope images of external surface of P3 bristles: Intact (Left); Isolated (Right). Scales are indicated (Photo: W. Geller).

2. Cinematographic Method

A cinematographic study was conducted using a high-speed camera (Photo-Sonics 4C; 250 frames per second) operated in three dimensions (left-right, forward-backward, and up-down) with fixed microscopic lenses. The dorsal side of a tethered *Daphnia magna* (Straus, 1820), measuring 3.7 mm in length, was glued (Bostic Super-glue 4-Cyanoacrylate) onto the tip of a rigid plastic sieve inside a 50 ml glass container filled with filtered lake water (0.45 μm filter paper, Bodensee, Germany). The internal water flow between the two transparent carapace valves was detected by a small drop of Indian Ink, injected with a superfine screw-thread injector near the animal's intake. The resulting film was analyzed through slow-motion projection, combined with a time-motion analyzer, of individual frame-

by-frame images (at 10 millisecond intervals) and magnified still photographs. Additionally, a visual indication of fluid penetration through P3 and P4, moving from the inner to the outer surface of these paddles, was observed.

3. Results and Discussion

What is the activation principle involved in the feeding mechanism of Daphnia? Sieving, Assembling or Dewatering.

Fluid dynamics definition of filtering action includes water flow through a sieve and particles larger than its mesh size retained, whereas cinematographic films denied water flow through P3 and P4 trunk limbs, mistakenly defined as filters. Therefore, food particle ingestion by Daphnia is redefined as an assemblance action. Food particles assemblance resembled the industrial dewatering action of algal and blood cells or oil droplets in micro-tubes [12]-[20]. The usage of Cinematographic technology implied a documentation of assembling [1]. The assembling mechanism was also partly suggested earlier [21] [22]. The cinematographic technology created a disputed response to the query: *Daphnia's* feeding mechanism is sieving or assembling? If “sieving”-the selection and efficiency of ingested particles are mesh-size dependent, which was denied by cinematographic research. Though the mechanism is probably controlled by fluid dynamics rules and other supportive factors of the particles' assembling process. Consequently, confirmation of the assembling mechanism deserves further investigation. The assembly mechanism recognition may spark a heated professional debate. Taking into account the long history of existence of the “sieving” mechanism paradigm, objections are expected. The more scientists are obliged to a sieving mechanism, the more difficult the change is to be accepted. The “assembling” hypothesis is a revolutionary change in the research field of the feeding mechanism in Daphnia.

3.1. Trunk Limbs Anatomical Structure

Recognition of internal water flow through the open space in between P3 and P4 limbs was confirmed by Cinematographic study [9]-[11]. The daphniids have 5 pairs of thoracic appendages (trunk limbs), of which P3 and P4 were restricted for particle abstraction [6]. The ultra-structure of these limbs consists of three components (**Figure 1**): 1) Setae; 2) Two rows of Setule located perpendicularly on each Setae; 3) Two rows of ultrafine knobs (bosses) on both sides of each Setule (**Figure 1**) [23] [24]. The interval between the setule is 0.6 - 0.7 μm , whilst the ultrafine structured knobs caused the free space volume between the setule to be even narrower. This structure in Daphnia creates tiny micropores (0.6 - 0.7 μm diameter), which researchers have attributed to the sieving device [10]. This structure was considered the sieving apparatus that retains food particles.

Daphnia employs two internal alternate micro-flows carrying food particles, of which one is directed in the space between P3 and P4 and the other is a trunk limb bypass towards the distal outlet exit [9]. The assumption of particles canny (stuck) by filter plates bearing setule bristles was concluded [6]-[8]. Nevertheless, it is cir-

cumstantial evidence but wasn't evidently confirmed by either photo or slow-motion running of cinematographic film. Thought, P3 and P4 structure was not confirmed as a functional network structure where every node is interconnected with every other node, forming a lattice-like pattern of connection. The bristles are separated from each other and the branched setules are totally free and individually isolated from the base to the tip. The documented functional anatomical structure of P3 and P4 [6]-[8] is not appropriate for a filter action. Sieving action includes a lattice mesh through which fluid flows and suspended particles are retained on its surface. Solid particles are removed by a perforated surface of filter substance that permits the fluid to pass through and solid particles larger than the filter mesh size are retained. P3 and P4 are employed as flexible solid paddles which suck water inside and press it outside while free setules are hovering inside the interlimb space. The sieving model defines this spaced volume as a "filter chamber" [6]-[8] which is full of fluid medium while collecting suspended solid food particles. Despite the medium flow through a sieve, it was wrongly defined as sieving. When P3 and P4 limbs are flapping backward, water is expelled posteriorly. Consequently, the two assumptive models, sieving and assembling, don't supply clear evidence on how the particles are attached or glued to the setule and smoothly disconnected when removed further towards the midgut.

3.2. Functional Properties of P3 and P4

Several assumptions were considered as mechanisms of particle attachment to the setule surface, such as chemical, electric and adhesion linkage, whereas the attachment of particles to the setule is not done by a sieving mechanism was confirmed. P3 and P4 are gleaners or gatherers that compile suspended particles, but not filters. The right physical term definition of the particle abstraction is therefore assembling. The dispute between filter and assembler definitions is not a semantic issue. Sieving (filtering) and assembling are physically dissimilar. Assembling is a process in which particles are not trapped or captured as a result of having a larger size than the mesh size; they are collected. The "sieving" definition was created as the result of anatomical structure interpretation, whilst the "assembling" definition resulted from cinematographic evidence confirmation supported by fluid dynamic rules analysis, which denies water flow through a filter structure. The up-downward beats alternate smoothly. P3 and P4 activate the rhythmic cycle of suspended driving it into the "filter chamber" and forwarded to the interlimb spaces and expelled posteriorly without any cross through a sieving structure [4] [9] [10]. Capillarity rules considerations [25]-[27] confirmed the irrelevance to internal water motions in *Daphnia*.

3.3. Internal Alternate Water Micro-Currents

The difference in energetic demands between assembling and filtration through micropores with a diameter of 0.7 - 0.6 μm or less, [4] [10] [28] is crucial because filtration requires excess investment. An increase in water viscosity and a decline

in Reynolds number are accompanied by an enhancement of operational energy demands. Moreover, sieving by P3 and P4 has the potential for fluid flow to undergo irregular disturbances or even create turbulent flow. Whereas, without interferences caused by sieving, a laminar flow is smoothly granted as an undisturbed microflow system, whilst sieving causes a stirring effect. Analytical evaluation of the Cinematographic film has indicated internal water flow velocity of 127 mS (milliseconds) along 36.25 μm route (L) (lengths of P3 and P4), which is 0.285 μm per 1 mS was estimated [9]-[11]. It is a fluid flow velocity associated with low Reynold Number (Re), which is a characteristic feature of Laminar flow. If viscous flow forces (resistance to flow) along the P3 and P4 pathway are enhanced, as predicted by sieving action, Re declines. Flow velocity reduction affected by sieving causes erratic turbulent flow and lower Re. Laminar flow enables optimization of the micro-water currents management, accompanied by the saving of metabolic energy. The operational mechanism of the trunk limbs is a rhythmic pulsated stroke beat that transfers water lumps through the “filter chamber” [6]-[8] from the proximal to the distal body part. Coordination is maintained between the two microcurrents, whereas filtering might cause system disturbances. Sieving activity, as fluid flow through a mesh, is an ecological disadvantage property. The sieving action of fluid flow through a mesh is an ecologically disadvantageous property due to the required energy investment. The outcome of functional replacement of sieving by the assembling method probably justifies a contribution of ecological compensation, creating an existing challenge for future research.

3.4. Feeding Mechanism Energy Demands

A filtering system comprise of a sieving device and a suspended particles medium flow. While in action, the developed pressure drop process continual require either the removal of retained particles or additional energy investment for pressure renewal. Consequently, enhanced (ΔP) and dropping alternate pressure cycling are predicted [14]. Nonetheless, slow motion of the cinematographic film and 10 milliseconds (mS) interval solid photos analyses deny pressure drop throughout the trunk limbs beat. A smooth in and out flow continuity along the internal space of P3 and P4 (“Filter Chamber” [6]-[8]) is confirmed. The inflow is caused by suction power (vacuum) during the downward beating of the limbs and the out-flow by maximal pressure during the upward beating.

Pressure drop increase per unit time as a function of particle size, density, and hygroscopicity loading in the Nucleopore filter was documented experimentally [20]. A ramification of the experimental result to natural internal conditions in *Daphnia* implies a decline of the suspended sieving flow velocity and efficiency and consequently particle ingestion rate. Correlation between mesh-size and ingested food particle-size by *Daphnia* was evidently accepted as the result of dimensioned anatomical structure analysis [2] [3] [24]. This total long-term acceptance was supported by an indirect indication done by solid SEM’s (Scanning Electron Microscope) photos not in action. Nevertheless, water flow through a

network mesh was neither observed nor documented. Clear evidence of water flow along the internal surface of P3 and P4 has been documented by a High-Speed Camera (250 frames per second), but operational sieving in action was not indicated. The result of P3 and P4 bristle size and spaces between them and retained particle sizes supported the interpretation of their correlation with ingested particle size. In other words, the smaller the bristles and the distance between each, the higher the efficiency of particle-bristle encounter and the assembling rate. It is now open for further research promotion for the discovery of created forces that enable particle adhesion onto the bristles of P3 and P4 trunk limbs. Are they physical or physico-chemical or organo-chemical traits or electrostatic energy factors, or other? The role of the particle-bristles surface area ratio is therefore critical. The smaller and finer the bristles are, the lower the energy investment and the higher the beat frequency. It is probably also affected by friction reduction. Sieving activity, as fluid flow through a mesh, is an ecologically disadvantageous property due to the required energy investment. The replacement of the interpretation of sieving by the assembling feeding mechanism also indicates an ecological benefit.

3.5. Filtration or Screening

Filtration is a method of separation that utilizes a permeable medium through which a suspension is passed. The permeable medium retains the solids and allows the liquid to pass through. Whereas screening involves passing the suspension through a screen with a specific pore size. The solid particles that are larger than the screen pore size are retained, while the liquid phase passes through the screen [14]. The two methods are inappropriate for *Daphnia*'s feeding mechanism. The industrial sieving flow-through rate determines how much medium solution is processed through the filter and is the key factor that determines the overall energetic investment. Thought, the filter flow-through rate is determined by the size of the screen openings. The size of the screen/filter opening is dependent on the particle size. Larger particle size will require larger openings, resulting in faster flow rates and a lower energy cost. The concentration of the particle size is an influential factor in the efficiency of the process. A high fine size particles concentration can result in blocking of the screen, while a low microalgal concentration can result in inefficient capture [14]. These considerations are due to the sieving/screening method but not with respect to the assembling mechanism. Industrial filtration requires a pressure drop to be applied across the system in order to force the fluid through the filter. The extent of pressure required for the medium determines which type of driving force is operated, gravity, vacuum, or pressure, of which only pressure is relevant to the feeding mechanism of *Daphnia* [1] [10]. A critical problem associated with the filtration/screening mechanism is that the adaptation of the so-called filter/screener industrial apparatus to the *Daphnia* mechanistic structure does not exist. The *Daphnia*'s feeding mechanism enable captivity of size varieties and concentrations that are fine enough to retain the fine and large particles in unchanged meshes.

3.6. Industrial and Natural Dewatering

Industrial filtration methods that operate under pressure or vacuum are suitable for recovering microalgal species with large cell size, but are inadequate to recover microalgal species with sizes approaching bacterial dimensions [19] and therefore inadequate for *Daphnia*.

The conditions leading to size-separation of suspended particles mixed in microfluids were experimentally investigated [16] [18]. The study of the flow-driven features resulted in a model of Deterministic Lateral Displacement (DLD). Microfluidic Systems conditions are leading to the size separation of suspended particles. The particles move along a principal direction in the microtube until a locked-to-zigzag transition takes place when the driving force reaches a critical angle. Filtering is not involved in the process of assembling particles abstraction and water sieving is needless. The ability to focus, separate and concentrate specific targets (suspended particles) in a microfluid is essential for the analysis of food items abstraction, where a myriad of different particles are presented as a size and composition mixture moving between P3 and P4 in *Daphnia*. It is suggested that within the feeding mechanism capabilities in *Daphnia*, filtering is not required for selective particle assembling and abstraction. Energy directing (Δp) to operate filtration through the interstitial spaces [28] will interfere with the entire system of water transmission. Ingested particles through water inputs must be assembled for further transport into the digestive tract for digestion. According to the classical interpretation, particle sorting and assembling were implemented by sieving, although fluid flow through the combs was speculated but never confirmed. The operation of P3 and P4 is a natural systems which utilize inertial forces, filtration, and adhesion mechanisms to assemble food particles.

Although fluid flow through the combs was never observed, filtration was concluded [7], but here it is instead concluded as “no filtration.” Observations of the similarity of “no flow” through the combs were misinterpreted as both filtration and no filtration, while the latter was clearly confirmed. The “no filtration” finding is supported by technological studies on particle assembly, which is driven by external energy sources [15]. Technological facilities confirmed that active systems use external energies—such as acoustic, electric, magnetic, and optical—to exert forces that displace cells for sorting in super-micro fluid currents [15]. In conclusion, the removal of food particles by sorting and assembly within the space between P3 and P4 in *Daphnia* is not maintained by sieving. As a result, this presents an open area for further research, where biological and physical traits overlap and intertwine. Ingested food particles must undergo dewatering for continuous feeding, with subsequent processing, grinding, and transfer into the midgut.

A similar obstruction is the industrial-scale processing of microalgae filtration for biofuel production. Harvesting of microalgae from diluted suspended fine particles solution creates an operational industrial difficulty during dewatering, as well as in *daphnia*, in case filtration is activated. Therefore, evolution preferred assembling and replaced filtration. Currently, there is no industrial technique of

dewatering microalgae that may result in a greater commercial efficiency of filtered algal biomass, which may have led to cost reduction and energy consumption. Filtering process rules affect *Daphnia* and the Industrial process is costly and requires invested energy and is algal cell size dependent. The natural evolutionary solution in *Daphnia* was particle assembly [14].

3.7. Evolutionary Perspective of Feeding Mechanism Development

Evolutionary progressive development promotes resiliency and diminishes ecological weakness. Solid walls of P3 and P4 support the improvement of ecological resiliency. The development of P3 and P4 functional usage has evolved towards resiliency and not fragility. The functional operation of P3 and P4 as “flexible solid walls”, followed by food particles assembling, improves animal resiliency. The definition of “solid flexible walls” [2] of P3 and P4 appendages is right, and the strainer is incorrect. The food particle abstraction process requires dewatering for further digestion. Because low Reynolds number (viscous flow) regimes prevail, the boundary layer around the setules within the “filter chamber” [6]-[8] may exceed their inter-setular distances and little or no flow takes place between these setules [21] [22]. The P3 and P4 function is as pumps [29] [30] and not as filters [31] [32]. Assembled food particles are collected not by sieving but are packed together. Created particle packages are pushed through the mouth to be ground by a pair of mandibles and forwarded into the midgut. The rate of package delivery from the “filter chamber” [6]-[8] towards the mouth and through the mandible into the midgut depends on suspensoid density and size: The higher the density and the particle size, the higher the rate of package delivery.

The “no filtration” model is supported by technological studies about particle assembling, which is operated by external sources of energy [15]. Technological facilities confirmed in active systems that exploitation of external sources of energy (acoustic, electric, magnetic, and optical) imposed forces to displace cells for sorting in super-micro fluid currents [15]. Conclusively, food particle abstraction by sorting and assembling within the space between P3 and P4 in *Daphnia* is not maintained by sieving. Consequently, it is an open gate for further investigations where biological and physical traits overlap and intermingle. Evolutionary progressive development promotes energetic resiliency and minimization of ecological weakness, and the assembling process is an exciting gate opening for future investigations.

Evolutionary development is commonly accepted as aimed at the optimization of ecological adaptabilities. The advanced progress trend of ecological adaptability of the improvement of P3 and P4 functions resulted in flexible solid wall creation. The evolutionary process of implementing improvement evolved towards solid walls, whilst the sieving function is a disadvantageous property. Evolutionary progressive development promotes resiliency and diminishes ecological weakness. Solid walls of P3 and P4 support the improvement of ecological resiliency. Evolu-

tionary development or functional ecology improvement evolved towards progressive adaptation of P3 and P4 through minimizing metabolic energy investment to achieve the highest efficiency of food particle abstraction. Therefore, the elimination of the sieving function from P3 and P4 usage in *Daphnia* was a compatible ecological process. The development of P3 and P4 functional usage has evolved towards resiliency and not fragility. The functional operation of P3 and P4 as “flexible solid walls” without sieving evolved, and the decline of energy investment was implemented. The improvement in energy balance enhances energy channeling to other organism activities. P3 and P4 sieving functions might deteriorate escape-ability and enhance vulnerability and refuge necessity, whilst eliminated sieving functions improve animal resiliency. P3 and P4 should therefore be termed assemblers and not filters. The definition of “solid flexible walls” [2] of P3 and P4 appendages is right and the strainer is wrong. An energetic wavy gradient is created by the limbs flapping stretch from the entrance to the exit of water into the cylindrical pipe space between P3 and P4. It is therefore hypothetically evaluated that along this gradient, additional perpendicular pressure for pressurized water into the micropores for filtering maintenance is a kind of disturbance. P3 and P4 are moving and create this energetic gradient within the carapace. Water will therefore move from the higher end of a gradient to the lower one in the described fashion that fits the fluid nature of the water [9]. The food particle abstraction process requires dewatering for further utilization. Water micro flow continues smoothly while particles are independently dewatered and assembled. The mechanism of particle dewatering is presently unknown, except that particles are dewatered and assembled and filtered migration through the micropores does not exist. Moreover, because low Reynolds number (viscous flow) regimes prevail, the boundary layer around the setules of the so-called “filter setae” may exceed their inter-setular distances [28] and little or no flow takes place between these setules [29] [30]. The P3 and P4 function as pumps [30] and not as filters [31] [32]. Assembled food particles are collected not by sieving but are packed together. Created particle packages are pushed through the mouth to be ground by a pair of mandibles and forwarded into the midgut. The velocity of package delivery from the setular spaces into the mouth and through the mandible into the midgut depends on suspension density, particle size and material composition trait: The higher the density and the particle size, the lower the velocity at which packages are delivered.

3.8. Assembling, Sieving (Filtering), Screening, Dewatering

1) **Assembling** is different from screening, filtration, and dewatering and most appropriate functional feature definition in the complexed *Daphnia* feeding mechanism. Dispersed (food) particles assembling is by definition of being assemble into one place (site, locus)—setule edges in our case.

2) **Screening** action is separation of particles by using a mesh, screen or a sieve being pass through stably fixed suspended solution, or inversely, fixed stable

whilst solution flow through it. Separating food particles from fluid solution. To sift through a mesh from fluid solution. Food particle separation out by sifting mesh/screen.

3) Dewatering-collective separated (food) particles from fluidic solution. Sieving, filtering or screening are different titles of similar action—a process used to separate particles by using a perforate surface. The mesh function is separating particles from the liquid. Definitions of particles entitled sieving, screening or dewatering are due to function similarity: particles collection from liquid flow through the mesh. The cinematographic methodology including frame by frame (10 mS intervals) of slow motion projection and solid photos visual observation enabled conclusion of denied sieving feature. A flow through of liquid solution through the mesh whilst particles are retained, captured, trapped or caught was denied. Sieving action maintain when solution flow through the mesh whilst assembling mechanism feature occur when solution-particles mixture, liquid and particles, flow beside and along P3 and P4 and come into contact with the setule. During particles-setule contact, not yet verified forces (chemical, electrostatic, geochemical, physical adhesion etc.) causing them to be attached. Such a dynamical construction ensure maintenance of continuous laminar smooth flow with low Re whilst sieving might create flow perturbation, increase Re and energetical investment. Flow laminarization decline Re whilst generating turbulence enhance Re.

3.9. Conclusive Remarks

Scientific blunders are not only inevitable but also contribute to scientific progress. It is predicted that defining the sieving trait of P3 and P4 as a blunder will probably stimulate further investigation. The impact of introducing a new scientific definition within the community is expected to face strong resistance, primarily from those who have much to lose [33]. Updated observations clearly justify the change presented in this paper. Possibly, the “sieving” definition will be categorized as a historical blunder [33]. The evolutionary development and function of the P3 and P4 trunk limbs in *Daphnia*—acting as “flexible solid walls”—integral to the assembly mechanism of food particle ingestion, likely enhances the animal’s resiliency. However, the status of this statement remains incompletely resolved and requires further investigation. Circumstantial evidence supports the long-standing classical view of P3 and P4 as “solid walls” [2]. This revised approach, while requiring additional research, confirms that these organs function as pumps rather than “strainers.” Collected food particles are packed into “packages,” which are pushed toward the mouth to be ground by the mandibles before being transported into the midgut. In conclusion, advancements in research regarding herbivorous zooplankton feeding mechanisms are anticipated. Further study into the specialized motion of food particles from the environment into the animal’s midgut is necessary. The historical tradition defined the feeding mechanism of *Daphnia* as sieving [6]-[8], suspected of “not only sieving” was documented [21] [22] and recently assembling was concluded [1]). Scientists settle on

three disputed conclusions, and 4th additional combination will open newly crucial research fields. Sieving model for the feeding mechanism of *Daphnia* was an unavoidable blunder. Filtering model was crucial for the scientific promotion. Moreover, sieving was genius blunder which made the scientific progress clearer. Thought, linguistic clarification is critical [33]: Scientific Mistake—a case caused by inadequate consideration or principle disregard; Scientific Error—a case of unintentioned consideration or deviation from the right way; Scientific Blunder—a case of lack of information, or attention, or failure, or negligence. Recognition of self-error is less common human being tendency than others mistake [33]. It was C. Darwin who stated in late 19th century that “even long-term existence opinions enabled being wrong and ignorance induce self-confidence”.

Scientific blunders are not only inevitable but also contribute scientific progress. It is predicted that the definition of the sieving trait of P3 and P4 as a blunder would be probably a stimulator for further investigation. The impact of a new scientific definition within the community is predicted to be intensively objected, mostly by those who have a lot to lose [33]. Renovated observations evidently justify the change presented in this paper. Possibly “sieving” definition would be categorized as a historical blunder [33]. It is commonly known that the more scientists are obliged to a certain idea the more difficult the change is accepted: Only dead fish go with the flow (M. Muggeridge).

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All the data generated and/or analyzed during performing this current study are available upon request.

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Conflicts of Interest

The author has no conflict of interest that may affect this article.

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